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THE HUMAN GALLBLADDER:
MICROSCOPIC AND MOTILITY STUDIES
IN NORMAL AND DISEASED

by

THOMAS MARTIN FEELEY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

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Dated October 28/83

A B S T R A C T

Altered gallbladder motility may be a factor in the etiology of cholesterol gallstone disease and in-vivo studies suggest there is an altered gallbladder response to cholecystokinin in humans with this condition. In-vitro human and animal studies suggest there is altered gallbladder response to histamine in cholecystitis and in the presence of lithogenic bile respectively. Although the histological features of calculous and acalculous cholecystitis are well documented it is unclear if these conditions are the same, or are in fact, 2 or more separate entities.

In this study the microscopic features of normal gallbladders and those with calculous and acalculous cholecystitis were studied and in-vitro motility was studied in the 3 gallbladder groups.

Human gallbladders removed because of suspected cholesterol gallstone disease were collected immediately following excision and transported to the laboratory in oxygenated Krebs solution. Sections were prepared for microscopic studies including measurement of muscle thickness and gallbladder strips were prepared for motility studies using standard organ bath techniques.

There was no distinguishing histological feature between acalculous and calculous cholecystitis, similar findings were present in both groups. Significant muscle hypertrophy

was present, without exception, in all gallbladders in both disease groups.

Gallbladder strips contracted in response to field stimulation and in the presence of atropine field stimulation caused relaxation which was inhibited by Propranolol but not by Practolol. Non adrenergic-non cholinergic inhibition was not seen in any gallbladder strip. Adrenergic receptor stimulation caused relaxation in all gallbladders and was antagonised by propranolol but not by practolol. α-adrenergic receptors were demonstrated in only a small number of gallbladders. Gallbladder sensitivity to histamine increased with increasing degrees of cholecystitis: the acutely inflammed gallbladder was 30 times more sensitive than normal. Sensitivity to cholecystokinin was the same in normal gallbladders and those with calculous and acalculous cholecystitis. However, in the mildly diseased group there was an increased contractile response when compared to normal and other diseased groups: the acutely inflammed gallbladder contracted very poorly in response to cholecystokinin. The cholecystokinin-like hormones, gastrin, pentagastrin and caerulein caused contractile responses in the gallbladder. Of the other hormones studied only substance P caused a contractile response; motilin, glucagon, secretin and others had no effect.

The similarity of the histological features of acalculous and calculous cholecystitis suggest that they are not separate disease entities. The increased sensitivity to histamine in acute cholecystitis may result in histamine

induced gallbladder spasm which may contribute to the pain associated with this condition. The results of this study show that the altered response by the gallbladder to cholecystokinin in cholesterol gallstone disease is probably due to altered contractility rather than altered sensitivity to cholecystokinin. Gastrin and Substance P may regulate gallbladder motility. That the gastrointestinal hormone motilin had no direct effect on gallbladder motility, suggests that its action in-vivo, is an indirect one.

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
CHOLESTEROL GALLSTONE DISEASE	1
REVIEW OF THE LITERATURE	5
ANATOMY	5
Neuroanatomy	6
PHYSIOLOGY	7
Bile Storage	7
Bile Concentration	9
GALLBLADDER MOTILITY	9
NEURAL CONTROL	10
Parasympathetic Nerves	10
Sympathetic Nerves	11
Non-adrenergic Non-cholinergic Relaxation	12
HORMONAL CONTROL	13
Cholecystokinin	14
Gastrin	16
Caerulein	18
Secretin	19
Vasoactive Intestinal Polypeptide (VIP)	19
Glucagon	20
Substance P	21
Motilin	22
Somatostatin	22
Neurotensin	23
Bombesin	23
Other Regulators of Gallbladder Motility	23
Histamine	23
Prostaglandins	24

PAGE

GALLBLADDER DISEASE 24
Chronic Cholecystitis 25
Acute Cholecystitis 26
Acalculous Gallbladder Disease 27
Motility of the Diseased Gallbladder 30
Calculous Cholecystitis 32
Acalculous Gallbladder Disease 33

THE PRESENT STUDY 37
OBJECTIVES 37
Studies on Guinea-Pig Gallbladder 38

MATERIALS AND METHODS 39
Collection of Gallbladders 39
Gallbladders from Organ-Donors 42
Normal Gallbladders 43
Microscopy 43
Histological Classification 45
Measurement of Muscle Thickness 45
Motility Studies 48
Preparation of Tissue 48
Chemicals and Drugs 49
Analysis of Data 51

RESULTS 54
MACROSCOPIC FINDINGS 54
MICROSCOPIC FINDINGS 54
HISTOLOGICAL CLASSIFICATION 54
Normal Gallbladder (n = 6) 55
Chronic Mild Cholecystitis (CMC; n = 54) 55
Acalculous Cholecystitis (n = 5) 62
Chronic Advanced Cholecystitis (CAC; n = 29) 67
Acute Cholecystitis (n = 17) 67
Mucocoele (Hydrops) 68

PAGE

MUSCLE THICKNESS 68
Normal Gallbladder 68
Cholesterolosis 68
Chronic Mild Cholecystitis 71
Chronic Advanced Cholecystitis 71
Acute Cholecystitis 71
Mucocele (Hydrops) 72

MOTILITY STUDIES 72
Base Tension 72
Spontaneous Activity 73

ACETYLCHOLINE 77

AGONISTS AND ANTAGONISTS OF ADRENERGIC RECEPTORS 80
Adrenaline, Noradrenaline 80
Isoprenaline 85
Beta-Blocking Agents 85
Phenylephrine 86

FIELD-STIMULATION 86

HISTAMINE 89
Histamine Receptor Characterization 90

HORMONAL STIMULATION 100
Cholecystokinin 100
Caerulein, Gastrin and Pentagastrin 113
Substance P 124
Vasoactive intestinal polypeptide (VIP) 127
Human gallbladder 127
Guinea pig gallbladder 132
Secretin and other hormones 141

SUMMARY OF RESULTS 142

D I S C U S S I O N 144
Normal Gallbladders 144
Cholecystitis 145
Acalculous Cholecystitis 146
Acalculous and Calculous Cholecystitis: The Same Disease? 148

PAGE

GALLBLADDER MOTILITY 149
Analysis of Responses 150
NON-HORMONAL REGULATION 151
Acetylcholine 151
Adrenergic Receptor Stimulation 152
Nerve Stimulation 152
Histamine 154
HORMONAL REGULATION 156
Cholecystokinin 156
Gallbladder Sensitivity and Contractility to CCK-OP 158
Gastrin, Pentagastrin and Caerulein 161
Vasoactive Intestinal Polypeptide (VIP) 163
Substance P 165
Secretin and other Hormones 166

CONCLUSIONS 169

REFERENCES 170

VITA 199

LIST OF TABLES

Table	Description	Page
1	Normal Gallbladders : Patients' Data, Results of Investigations, and Histology Scores.	44
2	Gallbladder Sensitivity to Histamine in normal and diseased states.	95
3	Sensitivities and Contractile Responses to Cholecystokinin-octapeptide in normal and diseased groups.	114

LIST OF FIGURES

Figure		Page
1.	PATIENT POPULATION	41
2.	HISTOLOGY SCORE SHEET	47
3.	EXPERIMENT RECORD	53
4.	GALLBLADDER DISTRIBUTION	57
5.	GALLBLADDER WALL	59
6.	CHRONIC MILD CHOLECYSTITIS	61
7.	GROSS CHOLESTEROLOSIS	64
8.	CHOLESTEROLOSIS	66
9.	GALLBLADDER WALL MUSCLE THICKNESS	70
10.	SPONTANEOUS ACTIVITY	76
11.	CONCENTRATION-RESPONSE CURVE FOR ACETYLCHOLINE	79
12.	NORADRENALINE AND PHENYLEPHRINE	82
13.	CONCENTRATION-RESPONSE CURVES FOR NORADRENALINE AND ISOPRENALINE	84
14.	EFFECTS OF FIELD-STIMULATION	88
15.	CONCENTRATION-RESPONSE CURVE FOR HISTAMINE	92
16.	RELATIONSHIP BETWEEN HISTAMINE SENSITIVITY AND DEGREE OF GALLBLADDER INFLAMMATION	94
17.	EFFECT OF HISTAMINE RECEPTOR ANTAGONISM	97
18.	CONCENTRATION-RESPONSE CURVES FOR 2-PYRIDYLETHYLAMINE AND 4-METHYL-HISTAMINE	99
19.	EFFECTS OF HISTAMINE H_2 RECEPTOR STIMULATION	102

Figure		Page
20.	TIME TO REACH MAXIMUM CONTRACTILE RESPONSE WITH CHOLECYSTOKININ-OCTAPEPTIDE	105
21.	RESPONSES TO CHOLECYSTOKININ-OCTAPEPTIDE CAERULEIN AND GASTRIN	106
22.	CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE	107
23.	CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE	112
24.	CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE	116
25.	RELATIONSHIP BETWEEN HISTOLOGY SCORE AND CONTRACTILE RESPONSE TO CHOLECYSTOKININ-OCTAPEPTIDE	118
26.	MUSCLE THICKNESS IN GALLBLADDERS USED FOR CHOLECYSTOKININ STUDIES	120,
27.	CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE, CAERULEIN AND GASTRIN/PENTAGASTRIN	123
28.	RESPONSES TO PENTAGASTRIN	126
29.	EFFECTS OF SUBSTANCE P	129
30.	CONCENTRATION-RESPONSE CURVE FOR SUBSTANCE P	131
31.	EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON GUINEA PIG GALLBLADDER	134
32.	CONCENTRATION-RESPONSE CURVES FOR VASOACTIVE INTESTINAL POLYPEPTIDE AND CHOLECYSTOKININ-OCTAPEPTIDE IN GUINEA PIG GALLBLADDER	136
33.	CONCENTRATION-RESPONSE CURVE FOR VASOACTIVE INTESTINAL POLYPEPTIDE IN GUINEA PIG GALLBLADDER	138
34.	EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON CHOLECYSTOKININ-OCTAPEPTIDE CONTRACTED GUINEA PIG GALLBLADDER	140

INTRODUCTION

CHOLESTEROL GALLSTONE DISEASE

Cholesterol-gallstone disease is a world-wide problem. Because of its commoness and the cost in lives and morbidity, as well as financial cost and the burden on health services, there is wide interest in its pathophysiology. As this is incompletely understood attempts to treat or prevent gallstones medically are at best speculative and thus far have met with very limited success.

The true prevalence of gallstone disease in the general population is unknown. Based on the Framingham Study (1), in the U.S.A. in 1972 there were 16 million people who had gallstones, in a female:male ratio of 3:1, and 5,000 to 8,000 deaths annually were attributed to gallstone disease. The prevalence of the disease varies enormously from country to country and in different ethnic groups: among the Pima Indians in Arizona, 70% of the women have gallstones by the age of 30 and 70% of the men by age 60 (2), whereas in Japan necropsy reports show an incidence of only 5% and the Masai tribe of Africa apparently do not have gallstones (3).
In Canada, in 1974, when the population totalled about 22.5-million, 89,000 cholecystectomies were performed - the world's highest rate for this surgical procedure (4); the ratio of female to male patients was 3:1, the same as in the U.S.A.

Knowledge of the pathophysiology of gallstones has completed one turn of a spiral since 1856, when Meckel von Helmsbach (5) suggested that bile stasis in the gallbladder was the primary etiological factor in gallstone formation. In 1932 Riegel et al (6) introduced the concept that the physicochemical properties of bile are important in the pathogenesis of gallstones, and Andrews et al. (7) later established the importance of the solubility of cholesterol in bile. During this time, alterations in the gallbladder wall, such as recurrent infection (8), were believed to be the initiating step in the chain of events leading to gallstones. In 1968 Admirand and Small (9) described the importance of bile salts and lecithin in solubilizing cholesterol and introduced the concept of lithogenic bile, in which the bile is supersaturated with cholesterol. Acceptance of this concept of lithogenic bile, and the observation that hepatic bile in patients with gallstones is lithogenic (10), diverted attention from the gallbladder as an etiological factor in gallstone disease. It was believed that the disproportion of cholesterol to bile salts and phospholipids (i.e., lithogenic bile), alone, was the cause of gallstones. In 1972, Dr. Small stated: "Within the next 10 years, a rational safe method for preventing cholelithiasis in high-risk groups and for disolving 'silent' gallstones will be developed" (11). There was extensive work on the enterohepatic circulation of bile salts; in 1976

Shaffer and Small proposed six categories of pathophysiological abnormalities that could lead to lithogenic-bile formation (12), and about this time evidence began to accumulate that pooling of bile salts in the gallbladder significantly altered hepatic bile composition (13). Then LaMorte et al. (14) summarized the evidence that altered gallbladder motility by altering bile salt circulation, may be an etiological factor in gallstone disease. That gallstones almost always form in the gallbladder and rarely in the bile ducts, and do not form after cholecystectomy, is circumstantial evidence that the gallbladder is necessary for gallstone formation.

There is some evidence to support the proposal of altered gallbladder motility in gallbladder disease. Lennon (15) showed that the gallbladder's response to histamine alters with increasing severity of cholecystitis and, further, suggested that the response to cholecystokinin may change with increasing severity of disease. However, this study had a major drawback - lack of normal gallbladder tissue for comparative studies.

Altered cystic-duct function may be an etiological factor in gallstone disease. In prairie dogs, animals in which a 0.4%-cholesterol diet results in cholesterol gallstone formation, Roslyn et al. (16) showed that the response of the gallbladder/cystic duct complex to cholecystokinin is altered in gallstone disease. Pitt et al. (17) reported increased

cystic-duct resistance in association with gallstones, and this was evident in the presence of lithogenic bile before the formation of gallstones.

If, indeed, there is increased cystic-duct resistance in association with gallstones, presumably it would cause bile stasis in the gallbladder. And so we have come full circle since this was first proposed by von Helmsbach in 1885. Meanwhile, although understanding of the pathophysiology of gallstone disease has increased remarkably in the last two decades, it is still not known whether gallbladder motility is altered and, if so, the basis of the alteration.

REVIEW OF THE LITERATURE

ANATOMY

The gallbladder is a pear-shaped hollow organ situated on the undersurface of the liver. It is connected to the extra-hepatic biliary tree by the cystic duct, through which bile flows to and from the gallbladder.

The wall of the gallbladder is composed of three layers - the inner mucosal, the middle fibromuscular, and the outer adventitial layer. The mucosal layer is composed of simple columnar epithelium which, when the organ contracts, is thrown into folds. This columnar epithelium lies on a thin, vascularized lamina propria (18).

In contrast to the muscle layer in the gastrointestinal tract, which is composed of well-defined circular (inner) and longitudinal (outer) layers, the muscle in the gallbladder wall is composed of a mesh of muscle bundles arranged in seemingly haphazard fashion (19) and interwoven with bundles of elastic tissue (20). This arrangement resembles the muscle in the muscularis mucosae of the intestine (19), a very thin muscle layer which underlies the intestinal mucosal layer. Muscle thickness, excluding the inter-bundle elastic tissue, in the canine gallbladder wall diminishes from the fundus to the neck (21), from an average of 90 μm at the fundus to 50 μm at the neck. In guinea-pig

gallbladder, muscle thickness was reported to be 80 μm (19) but it is not clear how the measurement was made. In the only report on normal human glalbladder (15), muscle thickness was recorded as 213 ± 50 μm; however, this was derived from measurements on four necropsy specimens, one of which was reportedly "mildly diseased".

The adventitial layer is composed of loose areolar tissue covered by the visceral peritoneum which covers that part of the gallbladder's surface which is not adherent to the inferior surface of the liver.

Neuroanatomy

The gallbladder receives preganglionic fibres from both vagus nerves and post-ganglionic sympathetic-nerve fibres via the splanchnic nerves and coeliac plexus (22). In studies (23,24) in guinea-pig, monkey, rat and human gallbladders, all authors describe three nerve layers: a ganglionic nerve 'plexus' that lies on the outer surface of the musculature (Auerbach's) of the gallbladder; a subserosal plexus that is associated with the adventitia of the cystic artery and its branches (23); and a submucosal plexus that lies in the lamina propria (Meissner's). Although some authors, (22,24) believe these to be three separate plexuses, others (23,25,26) describe a rich exchange of adrenergic fibres between the subserosal and Auerbach's plexus and believe they are part of the same

plexus. The ganglia are believed to be exclusively excitatory cholinergic (22,25), and the submucosal plexus is rich in adrenergic and acetycholinesterase-positive fibres. Cai and Gabella (23) reported finding very few nerve fibres running into the musculature of the gallbladder, whereas Baumgarten and Lange (26) postulated both direct noradrenergic (inhibitory) innervation of some muscle bundles and presynaptic inhibitory innervation of the excitatory cholinergic neurones similar to that reported in the intestine (27).

PHYSIOLOGY

The normal gallbladder performs three main functions: storage of bile, concentration of bile, and delivery of this to the duodenum when needed for the digestion of food.

Bile Storage

Traditionally, it was thought that all bile secreted by the liver was stored in the gallbladder to be delivered to the duodenum after the ingestion of food, but now it has been shown that, during fasting, 30% to 50% of secreted bile goes direct into the duodenum (28,29), by-passing the gallbladder. Bile flow into the duodenum and gallbladder is thought to be regulated primarily by the sphincter of Oddi (30,31). The gallbladder has the property of receptive relaxation; i.e., as its volume increases its intraluminal

pressure remains the same (32). Another possibly important factor in the regulation of gallbladder filling may be the reciprocal innervation between the gallbladder and the sphincter of Oddi (33), when the gallbladder contracts this sphincter relaxes. Although no other function has been described for this reflex, it may play some part in regulating gallbladder filling.

It was traditionally believed that no contractile activity occurred in the gallbladder during the fasting state, but recent very extensive studies by Itoh and co-workers (34-36) have demonstrated its occurrence and have identified four periods of gallbladder contractility between meals in conscious dogs. They recorded: 1, immediate postprandial contractions with a gradual increase in tone; 2, rhythmic contractions and relaxations; 3, a period of irregular contractions; and 4, an interdigestive contraction period in which strong regular contractions occurred in association with duodenal interdigestive migrating contractions. It is not known whether this contractile activity empties the gallbladder of bile or whether its function is to mix the bile to facilitate water and electrolyte absorption, as the composition of bile changes in each of the four periods (35). During the period of irregular contraction, the bile concentration decreased (35), indicating significant filling of the gallbladder at that time.

Our understanding of gallbladder motility during the fasting period is still incomplete and we know little of its regulation.

Bile Concentration

Early studies (37) of gallbladder physiology in dogs indicated rapid concentration of the hepatic bile, and absorption of 10 to 16% of the gallbladder volume per hour, up to 90% of gallbladder water being removed (38,39), whereas studies in guinea pigs (40) showed reabsorption of only 6% of the water. A human gallbladder containing gallstones can absorb up to 20% of intraluminal volume per hour (41), an absorptive capacity similar to that of the canine gallbladder. This absorption of fluid, together with secretion of mucus, increases bile viscosity. There is good evidence that lithogenic bile in the gallbladder stimulates the hypersecretion of mucus (42) and that gallbladder mucus gel is a nucleating agent for biliary cholesterol (42), but it is not known whether the increased viscosity of bile that results from this increased mucus causes bile stasis by slowing flow through the cystic duct.

GALLBLADDER MOTILITY

Gallbladder tone is regulated by neural (autonomic) and hormonal mechanisms. The autonomic contribution to gallbladder tone has been examined *in vivo* by nerve stimulation

and *in vitro* by field stimulation and the application of cholinergic and adrenergic agents.

NEURAL CONTROL

Parasympathetic Nerves

In vitro, stimulation of the gallbladder with acetylcholine or other parasympathomimetic agents increases tone (43) and electrical stimulation of the vagus nerve increases gallbladder pressure (44). It has long been realised that gallbladder-pressure increase due to vagal stimulation is not associated with evacuation of the gallbladder (44,45), but that when combined with subthreshold concentrations of cholecystokinetic hormones emptying does occur (46).

Numerous investigators have reported dilation of the gallbladder after total abdominal vagotomy (47,48), and some have described doubling of the gallbladder's resting volume after this procedure (48,49). Most authors believe this result is due to decreased gallbladder tone secondary to the vagotomy, indicating that the vagus is an important factor in the maintenance of tone. However, some contrary findings have been reported after this operation -- increased intra-gallbladder pressure (50) and dilation of the common bile duct (51). It is now generally accepted that the dilation which follows vagotomy may develop up to

one year afterwards, a lag-time that does not fit the concept of vagally-maintained gallbladder tone. A more likely explanation of the dilation lies in the gallbladder's property of receptive relaxation (32), which may enable it to enlarge as a result of increased resting pressure within the biliary tree. This would support the suggestion that vagotomy results in increased resistance at the sphincter of Oddi, a development first suggested by Williams and Huang (50).

There is evidence that the gallbladders of humans who have undergone vagotomy are hypersensitive to cholecystokinin stimulation (52), an occurance not found in animal studies (53).

The role of the vagus and its importance in gallbladder motility is not yet fully understood but it is believed that the parasympathetic nervous system interacts with hormonal stimuli in the regulation of gallbladder tone.

Sympathetic Nerves

In characterising the adrenergic receptors in feline gallbladder (54,55), Persson identified a small population of α-receptors, demonstrable only *in vivo* while the β-receptors were blocked, and showed that the predominant adrenoreceptor is the β-receptor. A larger population of α-receptors may be present in guinea-pig gallbladder (56), but none could be demonstrated in the canine gallbladder *in vivo* (57).

Stimulation of the splanchnic nerves relaxes the gallbladder (54,55), and the inhibitory effect of this stimulation is enhanced when gallbladder tone is increased by vagal or cholecystokinin stimulation (54,56). The application of both noradrenaline and adrenaline *in vitro* usually induced relaxation in the feline gallbladder (54), which also becomes more obvious when the gallbladder is precontracted; however, in studies on guinea-pig gallbladder *in vitro*, this caused a contractile response (56).

The significance of a predominantly adrenergic inhibitory innervation of the gallbladder is not known. It has been suggested that it plays a role in gallbladder filling (55,58); i.e. it may be responsible for the receptive relaxation that occurs in the gallbladder.

Non-adrenergic Non-cholinergic Relaxation

Since the turn of the century there have been reports of vagally induced contractile and relaxant responses which were resistant to atropine (59), responses that, in 1933, Henderson and Roepke (60) suggested might be induced by unknown transmitters. Since the discovery of adrenergic blockers there have been reports of relaxations resistant to these in almost all parts of the gastrointestinal tract, including the gallbladder.

This non-adrenergic neurotransmitter has not yet been identified with certainty, although a very strong case has been made for 'purines' (ATP) by Burnstock and his

colleagues (61). Of all the other suggested candidate transmitters, only vasoactive intestinal polypeptide is now believed to be a possible inhibitory agent (62).

Davison et al. (63) reported non-adrenergic inhibitory innervation in guinea-pig gallbladder and proposed that these nerves were purinergic (i.e. that, when stimulated, they release purine compounds such as ATP), a suggestion based on their finding that the nerve-mediated relaxations were closely mimicked by ATP. In the only other such study on guinea-pig gallbladder, however, Naughton (64) concluded that although non-adrenergic inhibition did occur it was not caused by purines.

In summary, it seems that non-adrenergic non-cholinergic inhibition does occur in the gallbladder and may be an important factor in gallbladder filling. To date, the transmitter has not been identified.

HORMONAL CONTROL

The primary determinant of gallbladder motility is hormonal (65). In 1928, Ivy and Oldberg (44) extracted from the mucosa of the upper small intestine of hogs a substance that, when injected intravenously, contracted the gallbladder and caused its evacuation. The authors named this substance cholecystokinin.

Cholecystokinin

Cholecystokinin (CCK) is present in the mucosa of the jejunum and to a lesser extent in the duodenum and ileum and is localized in a specific cell type of the APUD series, the 'I' cell (66). In the early 1960's cholecystokinin was isolated and purified by Jorpes and Mutt and identified as a linear polypeptide containing 33 amino acids (67). These authors further noted (68) that only the C-terminal octapeptide of the molecule is necessary for biological activity. This octapeptide was later synthesised (69) and shown to be three times more potent than the parent polypeptide, on a molar basis, in contracting gallbladder strips in vitro (70).

Until recently it was widely believed that the 33 amino acids constituted the principal cholecystokinin molecule released following the ingestion of a meal. However, with the ability to identify and measure individual molecular CCK forms in the plasma, there have been reports suggesting that the octapeptide is the predominant molecular form released into the circulation at that time (71-73): Dockray (71) and Walsh et al. (72) were unable to identify any CCK-33 or CCK-39 in plasma after a meal, and Hutchinson et al. (74) reported that CCK-8 accounted for more than 80% of the CCK immunoreactivity in guinea-pig myenteric plexus. After a fatty meal, the octapeptide may well be the predominant form released.

The effects of cholecystokinin on human gallbladder have been studied in vivo by several investigators (75-77). The effect, visually measured, is expressed as a percentage emptying of the gallbladder over a given time in response to a given dose. The gallbladder is outlined by the conventional contrast medium or radionuclide imaging (76,77), and evacuation is determined by planigraphic measurement of gallbladder size on serial radiographs (contrast method) or is calculated from recorded continuous counts made with a gamma-camera (imaging method); 30-45% emptying is regarded as normal. However, it has been noted that a bolus injection of the cholecystokinin results in decreased emptying, due to contraction of the gallbladder neck and thus obstruction to outflow (78).

In the few studies in vitro that have been reported (15,79), which were on diseased gallbladder tissue, there were dose related contractile responses to cholecystokinin. Assessment of CCK's effect per se has not been possible, as no studies of the effects of cholecystokinin on normal human gallbladder in vitro have been published.

The mechanism of action of cholecystokinin has been investigated in both human and animal preparations. The effect of CCK is not influenced by atropine, is not mediated by histamine receptors, is not prevented by α or β adrenergic blockade, and is not altered by tetrodotoxin (69, 80, 81). It is generally accepted that changes occur in the intra-

cellular (gallbladder smooth muscle) cyclic-nucleotide concentration in association with the action of cholecystokinin. An increased level of cyclic guanosine monophsophate (cGMP) after cholecystokinin stimulation, with little change in the concentration of cyclic adenosine monophosphate (cAMP), has been reported (82), and contractile responses induced by agents such as acetylcholine decrease the level of cAMP intracellularly. However, the literature reveals great divergence of opinion on the role of intracellular cGMP in the contractile effect of cholecystokinin. For example, Andersson (83) agreed that it was increased but concluded that this was not important to the cholecystokinin effect, whereas Longinov (84) stated that an increased intracellular concentration of cyclic-GMP is requisite to the contractile effect.

It has been established that the contractile effect of cholecystokinin is antagonised by dibutyryl cyclic-GMP (85,86) but the nature of this inhibition is not fully understood. Most authors believe it is competitive inhibition (85,87), but Miller et al. (86) proposed an interaction between the dibutyryl GMP and the cholecystokinin molecule.

Gastrin

There are structural similarities between cholecystokinin and gastrin (88): the carboxy-terminal pentapeptide amides are identical, and both have a tyrosine residue at

position 6 (gastrin) or 7 (CCK) - counting unconventionally from the carboxyl terminus. There are two gastrin molecules, one with sulphation of the tyrosyl residue (gastrin II) and one without (gastrin I). In all naturally occurring cholecystokinin, its tyrosine residue (position 7) is sulphated (71); this sulphation is essential to cholecystokinetic activity (69). In view of the similarities in structure it is not surprising that cholecystokinetic effects of gastrin have been reported. Vagne and Grossman (89) described one such effect which was equal for gastrins I and II; but on a molar basis, cholecystokinin was 22 times more potent than gastrin. In studies on rabbit and guinea-pig gallbladder in vitro, gastrin II was 20 to 33 times less potent than cholecystokinin (90). Cholecystokinetic effects have also been reported for pentagastrin, a synthetic unsulphated gastrin; thus, unlike the cholecystokinin molecule, gastrin may not require sulphation of the tyrosyl group for expression of its cholecystokinetic potency (89). However, Amer (90) reported gastrin II 20 times more potent than the unsulphated molecule, and found pentagastrin 10 times less potent than gastrin I. By contrast, several studies of human (91) and guinea-pig (92) gallbladder in vitro, and of opposum gallbladders in vivo (93), failed to demonstrate any contractile effect of gastrin I.

It is generally accepted that gastrin, in high concentrations, causes gallbladder contraction.

As concentrations required *in vivo* far exceed the minimum required to stimulate acid secretion, the effect may be pharmacological rather than physiological (89).

Caerulein

Caerulein, a decapeptide isolated from frog skin, has the same five carboxy-terminal amino-acid as gastrin and cholecystokinin. Like cholecystokinin, in its natural form caerulein has a sulphated tyrosyl residue at position 7 from the COOH terminal. Caerulein's effects on the smooth muscle of the gallbladder and gastrointestinal tract are similar to those of cholecystokinin (94). Its routine use has been recommended in the diagnosis of acalculous biliary disorders (95), as it causes spasm at the neck of the gallbladder in patients who have biliary dyskinesia. As in the cholecystokinin molecule, sulphation of the tyrosyl residue at position 7 is essential for full cholecystokinetic activity: when this is absent, potency has been reported reduced by a factor of 160 (96) and, in another study, by 75 times (97).

However, its de-sulphation minimally affects its ability to stimulate the secretion of gastric acid and pepsin (96).

Studies *in vitro* (80), and *in vivo* (94) have shown that, on a molar basis, caerulein is two (80) or ten times (94) more potent than cholecystokinin in contracting the gallbladder.

Secretin

Secretin's structure differs from that of the cholecystokinin family. This hormone is a member of the group that includes glucagon, vasoactive intestinal polypeptide, and gastric inhibitory peptide.

Lin and Spray (98) reported that both natural and synthetic secretin increased intra-gallbladder pressure in the dog, whereas other studies in vivo (99,100) have shown that secretin has no effect when given alone but augments the effect of cholecystokinin. A more recent study in vivo (92) demonstrated a slight relaxant effect of secretin on gallbladder basal pressure. It is now thought that the cholecystokinetic effect of secretin reported in some studies in vitro (43,91) probably reflected CCK contamination of the preparation.

The physiological significance of any secretin/cholecystokinin interaction is not known. As the concentrations of secretin needed to demonstrate augmentation of cholecystokinin's effect were far greater than required to stimulate pancreatic secretion, the response may have been pharmacological.

Vasoactive Intestinal Polypeptide (VIP)

VIP's most potent effect is on intestinal secretion (101) but it affects gastrointestinal motility, also. In the biliary tract this hormone is regarded as the most potent relaxant of basal and cholecystokinin-induced gallbladder

tone, *in vivo* (100, 102) and *in vitro* (103). VIP may be a mediator in the reported non-adrenergic inhibition of responses in the gallbladder and gastrointestinal tract (62).

It is known that nerve endings contain VIP (104,105). Increased levels of this hormone have been found in portal-vein blood after vagal stimulation (104), and Sundler *et al.* (105), using immunofluorescence histochemistry, demonstrated VIP in nerve endings in the gallbladder wall of several species, including man. Furthermore VIP from a VIP-producing tumour is believed to be the mediator in the disorder known as the W.D.H.A. syndrome, which consists in watery diarrhea, hypokalemia, and achlorhydra (106) and in which the gallbladder is commonly dilated (107). This is regarded as confirmatory evidence that VIP has a relaxant effect on basal gallbladder tone (104) in humans.

There have been no reported studies, *in vivo* or *in vitro* of the direct effects of VIP on human gallbladder.

Glucagon

Glucagon has structural similarities with secretin and VIP. In general, its action on the gastrointestinal musculature is inhibitory. Studies *in vivo* have shown that glucagon has a relaxant effect on canine (108) and human gallbladder (109); in the latter investigation, glucagon exerted its effect when given in the fasting state or after a fatty meal. *In vitro*, however, this relaxant effect could not be demonstrated in human (91) or guinea-pig (89) gallbladder.

The doses of glucagon required to demonstrate relaxation in vivo were much greater than necessary for glycogenesis, indicating that the effect probably was unphysiological.

Substance P

Substance P, another peptide, has been identified in the central nervous system, the nervous plexus of the intestine, endocrine cells of the intestinal mucosa, and human plasma (110), and most recently in nerve endings in the wall of guinea-pig gallbladder (23).

The effects of substance P are both variable and widespread. It inhibits and stimulates responses in arterial and venous smooth muscle, respectively, and stimulates intestinal smooth muscle (111). Its effect on vascular muscle appears to be direct, whereas in intestinal muscle the contractile effect is partly antagonised by atropine (112). In the intestine, substance P may act as an excitatory transmitter or neuromodulator. Findings in some motility disorders may support this. In patients with Hirschprung's disease, the proximal hyperactive intestinal segments were shown to contain more substance P than in controls whereas the aganglionic, inactive colonic segments contained much less than in controls (113). Furthermore, rectal biopsies from patients with Chagas' disease contained less than 50% of the amount found in a normal population (114).

Despite the interest in substance P's role in gastro-

intestinal motility, no studies have been published that examine its role in biliary motility.

Motilin

Motilin has no significant direct effect on gastrointestinal smooth muscle during the digestive stage but it is believed to aid regulation of the migrating myoelectric complex (MMC) (115). The MMC's are cyclic recurrent episodes of caudad-moving bands of strong contractions that move from the lower oesophageal sphincter to the terminal ileum.

Adrian et al (116) showed in vivo that motilin is almost as potent a cholecystokinetic agent as cholecystokinin, and speculated that it might be an important regulator of biliary motility. Several studies in vitro have failed to demonstrate a contractile effect of motilin on the gallbladder (117), but this does not discount the possibility that motilin may regulate biliary motility indirectly.

Somatostatin

Somatostatin may act with motilin in regulating the MMC (115). In the biliary tract, it has been reported to inhibit the gallbladder's contractile response to both intraduodenal acid and vagal stimulation (118).

Neurotensin

More than 90% of the neurotensin derives from the gastrointestinal tract. Excess levels of this hormone have been reported in association with the dumping syndrome (119); also, it may contribute to the modulation of gastric function (120), as studies *in vivo* have shown that neurotensin inhibits peristaltic activity (121). However, nothing is known of the effect of neurotensin on biliary motility.

Bombesin

First isolated from amphibian skin, bombesin-like immunoreactivity has since been demonstrated in guinea-pig myenteric plexus (74) and human endocrine cells. *In vivo*, bombesin has wide-ranging effects due to its potent releasing properties it releases both cholecystokinin (122) and gastrin (123), and its effects on the biliary tract are thought to be due to released cholecystokinin. There have been no reports of bombesin's effects on gallbladder smooth muscle *in vitro*.

OTHER REGULATORS OF GALLBLADDER MOTILITY

Histamine

Histamine is a potent contractor of guinea-pig (124), canine (125), primate (126) and human (15) gallbladder. However, its role in the regulation of biliary motility

is unknown, and extensive studies of the gastrointestinal tract have demonstrated either very modest or no effect of histamine on motility (127).

Both stimulatory (H_1) and inhibitory (H_2) histamine receptors, with dominant contractile effects, have been characterised in guinea-pig (124) and primate (126) gallbladder. In the guinea-pig, blocking the histamine H_2 receptors augmented the contractile effect of cholecystokinin (124).

Histamine receptors have not been characterised in the human gallbladder.

Prostaglandins

Prostaglandins have direct effects on gastrointestinal smooth muscle and may modulate its activity (128). They also act directly on the smooth-muscle cells of both the gallbladder and the sphincter of Oddi, producing contraction or relaxation at these sites (129), but it has not been established whether prostaglandins have a role in normal biliary motility.

GALLBLADDER DISEASE

The term 'gallbladder disease' is used here to describe a number of non-malignant conditions of the gallbladder, usually referred to as cholecystitis, some of which have little or no evidence of inflammation.

Chronic Cholecystitis

As gallstones are almost always seen in association with cholecystitis they were assigned a role in its pathogenesis until Boyd in 1923 (130), said he considered calculi incidental, not essential, to gallbladder disease. The mildest form of cholecystitis has been referred to as "chronic primary cholecystitis" (131) because the inflammatory process lacks an initial acute phase. Levine (20) referred to this as the hypertrophic stage; because of the hypertrophy of the mucosal and muscle layers. The normal mucosal folds are enlarged to become mucosal polyps, the muscle bundles become thickened, and intervening elastic tissue is less evident.

Usually there are only mild inflammatory changes, such as cellular infiltration of the wall and the formation of lymphoid follicles (20). A characteristic feature is the appearance of glands singly or in small groups in the mucosa and submucosa (20); normally, these glands are present in the area of the gallbladder neck only (20). Another frequent finding at this stage are Rokitansky-Aschoff sinuses (which are discussed later).

As the severity of the disease increases, the inflammatory changes, such as lymphocytic infiltration of the wall, ulceration of the mucosa, and fibrotic changes in all layers, become more evident and the 'polypoid' mucosa becomes atrophied. At the end stage of the disease, the gallbladder is a fibrous sac in which the mucosa is replaced by scar tissue.

Acute Cholecystitis

Acute cholecystitis rarely occurs de novo; it is usually superimposed on chronic cholecystitis. In a study of 150 gallbladders surgically removed because of cholecystitis, Levine (20) saw no case of 'acute cholecystitis', a finding that may reflect conservative management (non-operative) of patients with a clinical diagnosis of acute cholecystitis.

Several pathogenic factors have been proposed for acute cholecystitis, such as bacterial infection (132), occlusion of the cystic duct (133), and regurgitation of pancreatic contents into the biliary tree (134). In a recent study by Roslyn et al. (135), in prairie dogs, the combination of lithogenic bile and cystic-duct obstruction was demonstrated necessary to induce acute cholecystitis. Gallstones in the gallbladder were not requisite, and cystic-duct obstruction or lithogenic bile alone, did not cause acute cholecystitis. All cultures of bile from the gallbladders with acute cholecystitis were negative for both aerobic and anaerobic bacterial growth.

These findings strongly suggest that, at least in the experimental situation, acute inflammation of the gallbladder is not due to bacterial contamination and probably is a chemically induced cholecystitis. The latter is in agreement with the experimental findings of Thomas and Womach (136), who showed that an increase in bile-salt concentration within the gallbaldder when the organ is obstructed will produce the pathological picture of acute cholecystitis.

Acalculous Gallbladder Disease

Gallbladder disease in the absence of gallstones has long been recognised. In the 1920's writers disagreed on whether the prognosis after cholecystectomy is better in cholecystitis without stones (137-139) or with cholelithiasis (140-142): the former authors (137-139) considered cholecystitis without stones to be an early stage of cholelithiasis disease, and the latter group (140-142) believed that many normal gallbladders were excised and this distorted the results. Mackay, in 1933 (143), who reviewed the cases of 243 patients who had undergone cholecystectomy for "cholecystitis without stone", described four types of acalculous disease.

Several other classifications of acalculous cholecystitis have been proposed since then, mostly by radiologists who have based the differentiations on radiological and mciroscopical findings. Jutras, in 1960 (144), pointed out that microscopical changes in acalculous disease are not inflammatory in nature and proposed the term "hyperplasic cholecystoses" -- 'hyperplastic' implying hypertrophy of hyperplasis of normal tissue elements, and 'cholecystosis' indicating a pathological process distinct from inflammation. Jutras identified nine specific types of pathological changes in the gallbladder: cholesterolosis (two types), adenomyomatosis (three types), superficial neuromatosis, deep neuromatosis, elastosis, limpomatosis,

interstitial fibromatosis, pericholecystic fibromatosis and hyalinocalcinosis. Goldstein et al (145) in describing acalculous disease, used the term "hyperplastic cholecystosis" (without further classification) to describe gallbladder abnormalities; he included the 'cystic-duct syndrome', previously described by Cozzolino et al. (146), and spasm of the sphincter of Oddi. His classification was based on radiological findings, and he concluded that hyperplastic cholecystosis best described gallbladders that hyper-contracted in response to cholecystokinin. In 1983, Berk et al. (147) also using radiological techniques to classify gallbladder disease, proposed abandonment of 'hyperplastic cholecystoses' as a descriptive term and substitution of "cholesterolosis" and "adenomyomatosis" for routine use, arguing that the latter are radiologically identifiable as separate entitles.

The commonest microscopical abnormalities evident in acalculous cholecystitis are Rokitansky-Aschoff sinuses, cholesterolosis, and minimal lymphocytic infiltration. The extent of the infiltration considered necessary to constitute 'abnormal' is not clear - some is evident in most adult gallbladders. For example, Carman et al. (148) found only 17 normal gallbladders in a consecutive unrelated series of 5000 obtained at necropsy, and it is unlikely that more than a small proportion of these 'abnormal' organs had given rise to trouble during life; and Mackay (143), stated that microscopical changes probably are not significant

unless they are fairly severe.

Cholesterolosis, called 'strawberry gallbladder' because of its macroscopical appearance, is characterised by abnormal deposits of esterified cholesterol in the lamina propria. Rarely, the deposits are large enough to form nodules or polyps. The cholesterol is believed to be contained in macrophages and histiocytes which, on microscopy, appear as foam cells (149). Some authors believe that the condition is not associated with increased cholesterol content in the bile (150) or cholesterol gallstones (151), whereas others have reported the frequent co-existence of cholesterol gallstones and increased cholesterol content in bile (149).

Rokitansky-Aschoff sinuses are invaginations of the gallbladder's surface epithelium into its wall. Their pathogenesis is uncertain. These invaginations extend variable depths into the wall, in some cases extending through the entire muscle thickness. In one study, of 145 gallbladders excised because of benign biliary disease, these sinuses were found in 86% and calculi were present in 95% of these (152).

There are two further types of acalculous cholecystitis that clinically and microscopically are discrete entities: acute acalculous, and eosinophilic cholecystitis.

Acute acalculous cholecystitis has been reported in association with various unrelated conditions - trauma, surgery, systemic infection (153), burns (154), hyperalimentation (155), and as an acute complication

in the course of chronic systemic diseases such as diabetes mellitus, lupus erythematosus (156), and polyarteritis nodosa (157). Cholecystitis developing in systemic disorders probably is vascular in origin. The cause of acute inflammation in the former group is not known, though many factors have been suggested: anaesthesia, narcotics, analgesics, fasting in the post-operative period (158), multiple transfusions and sepsis (159). Microscopical examination reveals acute inflammation in the gallbladder wall; i.e. oedema, hyperaemia, erythrocytic extravasation, polymorphonuclear infiltration, and mucosal ulceration.

Few cases of eosinophilic cholecystitis have been reported (160, 161); in some, presentation was as a manifestation of eosinophilic gastroenteritis (162). Microscopical examination revealed diffuse infiltration of eosinophilic leucocytes throughout the entire thickness of the gallbladder wall.

Motility of the Diseased Gallbladder

There is evidence that altered gallbladder motility, by interfering with the normal enterohepatic circulation of bile salts, may be a factor in the pathogenesis of gallstone disease (14). Results of studies in animals in vitro and in vivo and in humans in vivo appear to support this.

In 1980, in experiments on prairie dogs, Roslyn et al. (16) showed that the response of the gallbladder-cystic-duct complex to cholecystokinin was altered in cholesterol-gallstone disease, and, in guinea pigs, Wise et al. (163) showed that a cholesterol diet increased the gallbladder's contractile response to histamine. Furthermore, the response to both cholecystokinin-octapeptide and acetycholine was decreased in gallbladders containing gallstones. In both these studies, however, sensitivity to agonists in the normal and diseased gallbladders was not studied. Davison et al. (164), who reported gallbladder contractility to cholecystokinin-octapeptide in associated with increased bile lithogenicity in ground squirrels, stated there was no change in gallbladder sensitivity to CCK-OP. However, in this study the lithogenic index did not reach 1 and gallstones did not form. In the only such study on diseased human gallbladder in vitro Lennon (15) suggested that the sensitivity of the diseased gallbladder to cholecystokinin altered with the disease state (mild and advanced chronic, and acute cholecystitis), but unfortunately he did not study normal gallbladder tissue.

Studies on human gallbladder in vivo have yielded conflicting reports on the sensitivity of the diseased organ to cholecystokinin. Such studies were on two groups of patients, those with cholelithiasis and those who have acalculous gallbladder disease.

Calculous Cholecystitis

Maudgal et al. (165), using standardized oral cholecystography to measure gallbladder emptying, demonstrated increased emptying in gallstone patients; they also measured the concentration of plasma cholecystokinin after a meal and found it did not differ between gallstone patients and healthy controls. In a further study by the same group (166) it was shown that the patients who had gallstones had increased sensitivity to exogenous cholecystokinin. Thompson et al. (167), who used Ultrasonography to measure changes in gallbladder volume, divided gallstone-containing gallbladders into "contractors" and "non-contractors": the contractors responded normally to meals but the non-contractors did not. Also, in measuring plasma cholecystokinin levels, these investigators found that the concentration was less in contractors than in healthy controls. They thus concluded that the gallbladder's sensitivity to cholecystokinin was increased in this 'contractor' group, and proposed two distinct groups of gallstone patients: one with increased sensitivity to cholecystokinin and one in which there was diminished gallbladder motility. Further contrasting results were reported by Fisher et al. (168). Using Gamma-emitting technetium (^{99m}Tc-HDA) to outline the gallbladder they showed that emptying in response to a standard meal was diminished in patients with gallstones. Fisher et al. (168)

further demonstrated a normal response by stone-containing gallbladders to cholecystokinin, whether this was infused or given as a bolus.

In summary, nearly all investigators agree that the gallbladder's response to cholecystokinin is altered in gallstone disease, the majority believing that this alteration is increased sensitivity to the hormone.

Acalculous Gallbladder Disease

Only a small percentage of cases of acalculous disease can be detected with standard oral cholecystography. If the cholesterol deposits are large enough, the cholecystogram depicts round fixed radiolucencies in the lumen of the opacified gallbladder (144). Rokitansky-Aschoff sinuses can be identified if they maintain a patent communication with the lumen and are large enough to create visible collections of contrast material adjacent to the lumen of the gallbladder (168).

Several authors have stated that the administration of cholecystokinin in association with cholecystography, which supposedly identifies abnormal motility, is an accurate tool for diagnosing acalculous gallbladder disease (145,146,170-175). However, there is lack of consensus as to what constitutes a 'normal' or 'abnormal' response by the gallbladder to cholecystokinin infusion, an issue that is further confused by the association of different gallbladder responses with apparently different types of acalculous disease. Goldstein's

classification (145) of acalculous gallbladder disease was based on the organ's response to cholecystokinin; the cystic-duct syndrome (obstruction of the duct) is associated with severe impairment of gallbladder emptying sometimes evidenced by the gallbladder's assumption of globular shape, and minimal filling of the bile ducts; partial obstruction of the sphincter of Oddi is associated with poor gallbladder emptying and incomplete filling of the common bile duct, with reflux of dye into the hepatic ducts; and hypercontraction of the gallbladder is associated with the hyperplastic cholecystoses. However, there is overlap of the histological appearances in the three groups, in that gallbladder disease, cholecystitis, and cholecystosis are present in almost all patients in the above three groups.

Nora et al. (170) described four findings with cholecystokinin cholecystography that indicate gallbladder disease: 1, duplication of symptoms (biliary pain); 2, abnormal local contractions in the neck or body of the gallbladder; 3, prolonged visualisation of the gallbladder; and 4, abnormal change in volume (a decrease of less than 50% within 20 minutes). They described "kinking" and "compression" of the cystic duct, and stated that histological examination revealed changes consistent with diagnosis of chronic cholecystitis in all of the gallbladders. Nathan et al. (175), who studied the gallbladder's response to cholecystokinin in 141 patients and 142 healthy controls, considered that recurrence of the patients' typical pain, spasm of the

gallbladder, and reflux of dye into the hepatic ducts are good indicators of gallbladder disease - but reported cramps in the right upper quadrant in 8% of the controls, mild reflux of opaque contrast medium into the hepatic ducts in several, and spasm in some. In contrast to many other authors, they stated: "The amount of speed and contraction alone are not significant". Furthermore, Valberg et al. (172) reported some controls in whom the gallbladder assumed globular shape in response to cholecystokinin; this is regarded by most authors as pathognomonic of disease. Valberg et al. believe that typical pain in response to cholecystokinin is the only good indicator of gallbladder disease whereas Dunn et al. (176), in a study of 74 patients and 44 healthy controls, reported cholecystokinin-induced gallbladder-like pain in 27% of controls as well as in 65% of patients in whom biliary colic was thought possible. Also, the radiological reports (mean of grading by three radiologists) recorded an abnormal response by the gallbladder to cholecystokinin in 28% of the controls as well as in 31% of the patients. Valberg's group therefore concluded the cholecystokinin cholecystography is not helpful in diagnosis or in management of the patients when acalculous gallbladder disease is suspected.

In an experimental study Goldberg et al. (177) carried out cholecystokinin-cholecystography in dogs who had chemically induced cholecystitis; no change in contractility could be demonstrated in chronic disease

but a tendency to increased contraction of the gallbladder was noted in acute cholecystitis.

In summary, the infusion of cholecystokinin has been reported to give rise to pain and to various abnormal responses by the gallbladder such as assumption of globular shape, hypocontraction (prolonged visualisation radiologically) and hypercontraction, in acalculous gallbladder disease. However, each 'abnormal' response has been reported to occur in apparently healthy controls, also.

THE PRESENT STUDY

Although the histological changes associated with calculous gallbladder disease have been described in detail, the histological/morphological changes in acalculous disease have not been well defined. Also, although muscle hypertrophy in association with gallstone disease has been well documented, the degree of the hypertrophy and its natural history have not been studied.

There is circumstantial evidence that altered gallbladder motility is an etiological factor in gallstone disease. Furthermore, several studies have indicated an altered response by the gallbladder to cholecystokinin in gallstone disease, *in vitro* and *in vivo*, and in acalculous gallbladder disease *in vivo*. However, these changes in response have not been demonstrated conclusively and the nature of the altered response is not known.

With these facts in mind, the objectives of the study were as follows:

OBJECTIVES

In human gallbladders:

1. To characterise changes in the wall in acalculous and calculus disease.
2. To measure and compare muscle thickness in normal gallbladders, in acalculous disease, and in calculous cholecystitis of various degrees of severity.
3. To characterise the gallbladder's response to neural

stimulation.

4. To characterise the response to muscarinic-receptor stimulation.
5. To characterise the response to adrenergic-receptor stimulation.
6. To compare the response to histamine stimulation in normal gallbladders and in various degrees of cholecystitis.
7. To characterise histamine receptors in the gallbladder.
8. To characterise the response to cholecystokinin.
9. To compare contractility and sensitivity to cholecystokinin in normal gallbladder, in acalculous disease, and in calculous cholecystitis of various degrees of severity.
10. To characterise the response of the gallbladder to gastrointestinal hormones.

Ideally all studies should have been carried out on nromal and diseased gallbladders. However, as the number of normal gallbladders in the study are small, they were used for cholecystokinin and, where possible, histamine sensitivity studies. Histamine studies were believed to be important as Lennon (15) had shown changing sensitivity to histamine with increasing severity of cholecystitis but had been unable to study histamine sensitivity in normal gallbladders.

Studies on Guinea-Pig Gallbladder

During the study, unexpected results were seen with some of the agents studied. Therefore, to establish the agents' potency, it was necessary first to record their effects in a proven experimental model.

MATERIALS AND METHODS

Human gallbladders were collected immediately following excision for proven or suspected gallstone disease at the following city of Edmonton hospitals: The University of Alberta, the Charles Camsell, the Misericordia and the Royal Alexander. Permission to collect the gallbladders was received from the surgeon and the senior pathologist. Usually the gallbladder from the earliest rostered cholecystectomy was collected. Every effort was made to collect gallbladders removed which did not contain gallstones. With the co-operation of the surgeons and residents in the above hospitals I was informed of planned cholecystectomies in patients with negative or inconclusive radiological investigations.

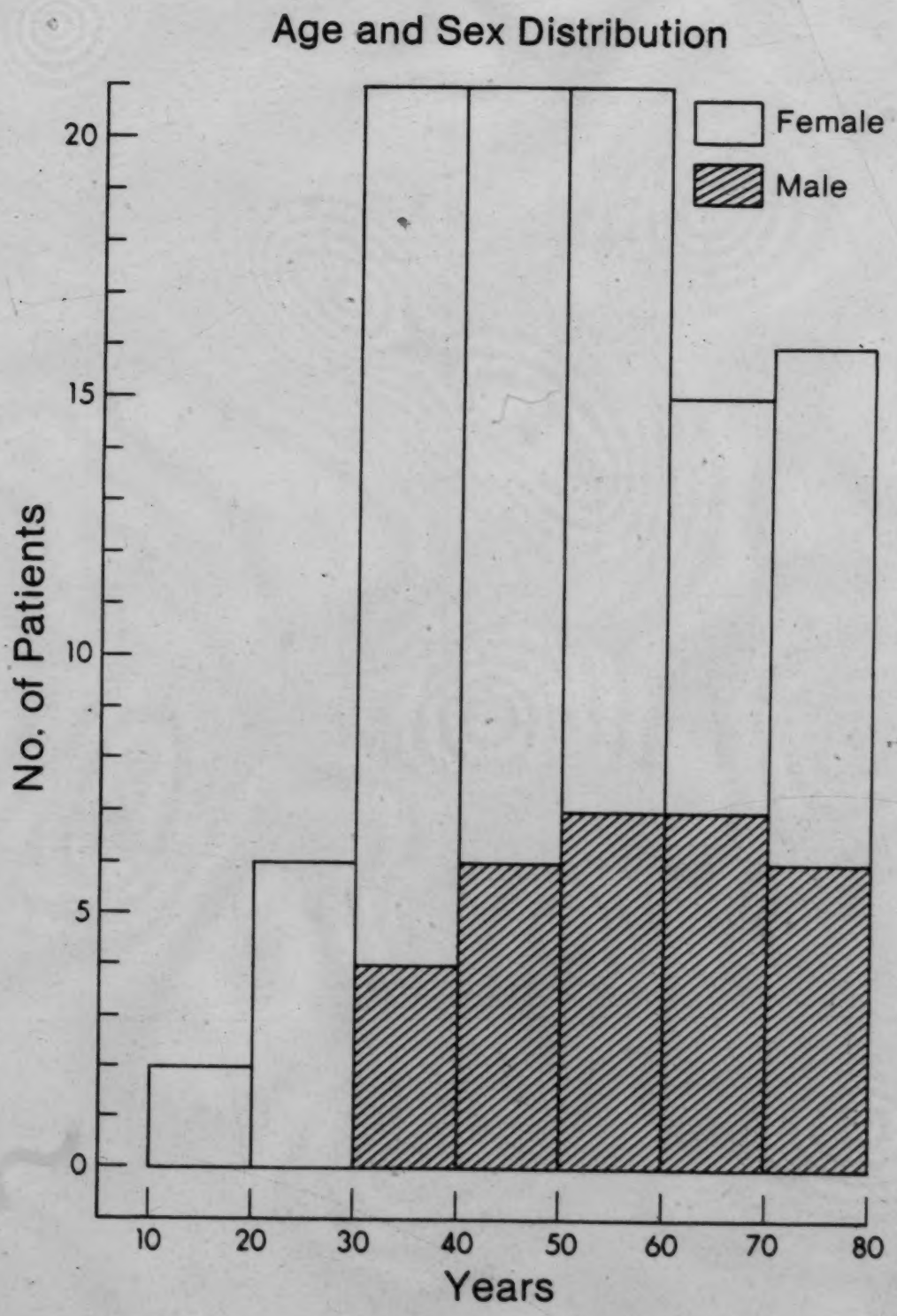
A total of 110 gallbladders were collected, four of which were severely diseased and traumatised peroperatively and were therefore considered unsuitable for the study. The age and sex distribution of the 106 patients whose gallbladders were used in this study is shown in Fig. 1. There were 75 female and 31 male patients, i.e. a female to male ratio of 2.4:1. The overall average age was 54 years; the average for the women was 53 and of the men was 56 years.

Collection of Gallbladders

Prior to cholecystectomy, clinical data and results of laboratory and radiological investigations were recorded.

FIGURE 1 : PATIENT POPULATION

Histogram showing age and sex distribution of the 106 patients whose gallbladders were used in this study.


Age and Sex Distribution
Female
Male
No. of Patients
0
5
10
15
20
10
20
30
40
50
60
70
80
Years

The appearance of the gallbladder and other abnormal abdominal findings were recorded per-operatively. Immediately following excision the gallbladder was immersed in oxygenated Krebs' solution and transported to the pathology laboratory. It was examined macroscopically by the duty pathologist who recorded, and advised the surgeon of any unexpected macroscopic abnormalities. The gallbladder was opened longitudinally and its contents were noted, including the number, type and size of calculi. A section (3.0 x 2.5 cm) was excised from the anterior wall of the gallbladder body and transported to the laboratory in oxygenated Krebs' solution.

Gallbladders from Organ-Donors

In order to draw significant conclusions from this study it was realised that it would be important to study normal gallbladder tissue. As there is a renal transplantation unit in the University of Alberta Hospital, it was hoped that normal gallbladders could be obtained from organ-donors. It was hoped that gallbladders collected in this fashion would supplement the small number of normal gallbladders excised because of suspected gallstone disease. Following submission of a study protocol permission was granted by the Ethics Committee of the University of Alberta Hospital to excise the gallbladders from these patients after the removal of other organs for transplantation.

No gallbladders became available from the transplantation unit during this study.

Normal Gallbladders

Six normal gallbladders were obtained surgically; all had been excised because of suspected gallbladder disease. The clinical and laboratory data on the 6 patients is shown in Table I. Gallbladders were classified as normal if the following criteria were met:

1. No calculi in gallbladder or extraheptatic duct systems.
2. Macroscopically normal wall; no cholesterolosis.
3. Microscopically normal wall; i.e. histology score less than 3, (see histological classification).

Microscopy

Immediately following arrival at the laboratory the gallbladder section was transferred to a large bath containing Krebs' solution which was continuously oxygenated with carbogen (95% oxygen and 5% carbon dioxide). A small section (1 x 0.5 cm) from the specimen was processed for histology. This was fixed to cork, mucosal surface uppermost, and placed in a specimen bottle.

All histology samples were fixed for 24 hours in 10% neutral buffered formalin, embedded in paraffin (Tissue-prep; Fisher Scientific Ltd., Edmonton) at 56.5 degrees centigrade, and sectioned transversly at 5 to 8 uM. The sections (4-6) were mounted on slides and stained with haematoxylin and eosin. Further sections from some gallbladders were stained with trichrome using Gomori's one-step method.

TABLE 1 : Normal Gallbladders‡ : Patients' Data, Results of Investigations, and Histology Scores.

SEX	AGE, YR.	SYMPTOMS & CURRENT CONDITIONS	LABORATORY DATA	RADIOLOGY REPORTS*	HISTOLOGY SCORE +
M	60	1 episode (1 hr) of epigastric pain 6	normal	OCG: non-functioning gallbladder (not repeated)	1
F	74	bilateral upper quadrant pain; chronic renal failure; polycystic kidneys	urea↑ creatine↑	US: gallstones	1
F	37	lifelong fatty-food intolerancy; 1 episode of pain in right upper quadrant	normal	OCG: normal gallbladder US: normal gallbladder	1
F	31	3 episodes of epigastric pain in 3 weeks	normal	OCG: normal gallbladder US: gallstones	1
F	55	2 episodes of pain in right upper quadrant	SGOT↑	US x 2: gallstones	0
M	45	recurrent episodes of epigastric pain, former 'heavy-drinker'	amylase↑	OCG: non-functioning gallbladder. ERCP: filling defects in gallbladder US: normal gallbladder	1

*OCG: Oral cholecystography, US: ultrasound of gallbladder.
+Scores less than 3 were considered normal.
‡At operation these gallbladders contained no gallstones and the biliary tree was normal.

Cover slips were applied with Histoclad (Clay-Adams) mounting medium to all stained slides.

The slides were coded, mixed and examined without knowledge of the motility-study results. All slides were examined independently by a second observer. Except for a small number of slides there was very good agreement in the histological scoring of the slides by both observers. Slides about which there was disagreement were examined by an independent third observer.

Histological Classification

The gallbladders were classified histologically using a modification of the method described by Lennon (15). A score was assigned to each component which contributes to 'histological' cholecystitis; e.g. inflammatory cell infiltrate, edema, and red-blood-cell extravasation. Each component was scored 0 if absent, 1 if mild or moderate, and 2 if extensive (Fig. 2), and the score was totalled for each sample. If the score was less than 3 the gallbladder was classified as normal; scores 3 - 5, 6 - 10, and 10+ were classified as chronic mild, chronic advanced, and acute cholecystitis, respectively.

Measurement of Muscle Thickness

Muscle thickness was usually measured in sections stained with haematoxlyn and eosin. In a few sections (all acute cholecystitis) in which the muscle did not stain well,

FIGURE 2 : HISTOLOGY SCORE SHEET

Copy of the histology score sheet used for histological classification. (Modified from that used by Lennon (54)).

TRANSVERSE SECTION

CODE NO. ____

DATE ____

	SCORE	NOTES
MUCOSA (INCL. LAMINA PROPRIA)		
CHOLESTEROSIS		
INFILTRATE: LYMPHOCYTIC		
NEUTROPHILIC		
EOSINOPHILIC		
PLASMA CELL		
RBCs (EXTRAVASATION)		
EDEMA		
ROKITANSKY-ASCHOFF SINUS		
MUSCLE		
HYPERTROPHY		
INFILTRATE: LYMPHOCYTIC		
NEUTROPHILIC		
EOSINOPHILIC		
PLASMA CELL		
RBCs (EXTRAVASATION)		
FIBROSIS		
EDEMA		
SEROSA		
INFILTRATE: LYMPHOCYTIC		
NEUTROPHILIC		
EOSINOPHILIC		
PLASMA CELL		
RBCs (EXTRAVASATION)		
FIBROSIS		
EDEMA		
TOTAL SCORE		

further sections were stained with trichrome which stains connective tissue and muscle green and pink respectively. Using an eye-piece micrometer (10 x objective) muscle thickness was measured in units, which when multiplied by 0.165 gave a result in microns (μm). Measurements were taken at 20 points or more on transverse sections of each specimen. In computing the muscle thickness the width of the connective tissue interspersed between muscle bundles was excluded from the measurement. The mean muscle thickness was then calculated for each of the 106 gallbladders.

Motility Studies

Preparation of Tissue

The gallbladder tissue was loosely pinned to a paraffin base in the bath containing the Krebs's solution. The serosal layer was dissected from the tissue which was then divided into longitudinal strips 3 x 10 mm. One end of each strip was attached by a fine silk ligature to a platinum hook at the bottom of a 5ml organ bath; the other end was attached by a similar ligature to an isometric force-displacement transducer, (FT-03C; Grass Instrument Co. Quincy, Mass.). The organ bath contained Krebs' solution maintained at 37°C, oxygenated with 95% O_2 and 5% CO_2 which was continuously bubbled through the solution in the organ bath and in the Krebs' solution reservoir.

The tension in the strips was initially set at 0.5 g;

when it altered spontaneously it was reset at this level. Preliminary studies had shown that a base tension of 0.5g was most suitable, giving consistent responses to repeated stimulation with various agonists. The strips were allowed one hour to equilibrate, being washed intermittently with Krebs' solution.

Recordings were made on a Beckman Polygraph Recorder: The Krebs' solution (pH 7.4) had the following composition (mM): NaCl, 116; KCl, 5.4; CaCl, 2.5; MgCl, 1.2; NaH_2PO_4, 1.2; $NaHCo_3$, 22; and D-glucose, 11.2.

Chemicals and Drugs

Drugs used and their sources were as follows: acetylcholine chloride, atropine sulphate, adrenaline HCl, histamine diphosphate, diphenhydramine, indomethacin, bombesin, caerulein, cholecystokinin-octapeptide, gastrin I, glucagon, motilin, pentagastrin, substance P octapeptide, secretin, vasoactive intestinal polypeptide, vasopressin, neurotensin, dibutyryl guanosine 3', 5' cyclic monophosphate, and tolazoline (Sigma Chemical Co., St. Louis); tetrodotoxin and somatostatin (Carbiochem); practolol (I.C.I.); secretin (Montreal Quebec); vasoactive intestinal polypeptide natural (Karolinska Institute, Stockholm) and synthetic (Peninsula Laboratories, Belmont CA); Pentavlon (Pentagastrin), Ayerst Laboratories, Montreal); phentolamine HCl (donated by Ciba-Geigy); 4-methylhistamine dihydrochloride, 2-pyridylethylamine dihydrochloride, cimetidine HCl (donated

by Smith, Kline and French, Canada Ltd.); impromidine trihydrochloride (donated by Smith, Kline & French Research Ltd.).

Fresh stock solutions of drugs to be used were prepared each day and serial dilutions were made. Concentrations were usually expressed as molar; one preparation of cholecystokinin and one of secretin, and vasopressin, were expressed as units per millilitre. All substances except indomethacin and pentagastrin (Sigma) were soluble in water. Indomethacin was dissolved in equimolar Na_2CO_3 solution and neutralized with HCℓ. Pentagastrin was dissolved in dimethylsulphoxide (DMSO 50%).

The effect of each agonist was tested on at least 2 strips from each of at least 10 gallbladders; starting with the lowest drug concentration, increasing concentrations were added until the maximal response was achieved. After the maximal effect for each concentration had been reached, the bath was washed out by overflow of fresh Krebs' solution; the tissue was allowed to return to base level, being washed out at least once every 10 minutes. The interval between drug application was at least 15 minutes, being longer if the tissue was slow to return to base tension.

When the effects of antagonists (e.g., indomethacin, cimetidine and diphenhydramine) on agonists were being assessed, the agent was made up in the reservoir of Krebs' solution in the required concentration, usually at the

beginning of the experiment; in these studies, 'time-control' strips (i.e., without the antagonist) were always studied at the same time. When synergistic effects were studied, one agent was added before, with, or at the point of maximal effect of, the other.

Analysis of Data

For each agent tested, the contractile response for each concentration was recorded, and was plotted as a percentage of the maximal response (Fig. 3) for that drug in that strip, and the mean response (grams and %max) was calculated for each concentration in strips from each gallbladder. A concentration response curve was constructed for each gallbladder, and from this the ED_{50} (the concentration required to cause half the maximal response) was calculated. The mean ED_{50} (with 95% confidence limits) was calculated for each drug in each disease group. When calculating the contractile response (g) and muscle thickness (μm), the mean, standard deviation, and standard error of the mean were calculated. Statistical analysis was the Student's t-test. P values of 0.05 or less were considered significant.

FIGURE 3 : EXPERIMENT RECORD

Copy of the form used to record experimental data. It illustrates the method used to record the sites from which strips for motility and histology studies were taken. Individual contractile and relaxant responses were recorded and were also expressed as a percentage of the maximum response by that strip to that agent.

EXPERIMENT NO. _____ DATE: _____

AGENTS

1. _____
2. _____
3. _____
4. _____

NAME: _____
AGE: _____ SEX: _____

CLINICAL DETAILS: _____

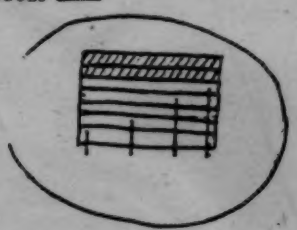
LAB DATA: _____

GALLBLADDER WALL: _____

CALCULI: _____ SOLITARY; _____ MULTIPLE.
OTHER INFO. _____

HISTOLOGY

MOTILITY STUDIES



SPONTANEOUS ACTIVITY

	NIL	FAIR	GOOD
A			
B			
C			
D			
E			
F			
G			
H			

MOTILITY DATA:

AGENT: _____

CONCENTRATIONS

TISSUE	CHANNEL	Response
A	1	GRAMS
		% MAX
B	2	GRAMS
		% MAX.
C	3	GRAMS
		% MAX.
D	4	GRAMS
		% MAX.
E	5	GRAMS
		% MAX.
F	6	GRAMS
		% MAX.
G	7	GRAMS
		% MAX.
H	8	GRAMS
		% MAX.

RESULTS

MACROSCOPIC FINDINGS

At operation 16 gallbladders were normal to inspection and palpation. The remainder were obviously diseased to various degrees with thickening and adhesions to surrounding structures. There were 5 mucoceles and there were no gangrenous gallbladders. Twelve gallbladders did not contain calculi; one of which was thick-walled and had a very wide cystic duct and furthermore there were calculi in the common bile duct, an indication that there had been stones in this specimen. Multiple calculi were present in 74 gallbladders and in 20 there was a solitary calculus. Eleven gallbladders had cholesterolosis of varying degrees, 6 of which also contained calculi. The five gallbladders with cholesterolosis and no calculi were macroscopically normal and per-operative cholangiography was also normal.

MICROSCOPIC FINDINGS

HISTOLOGICAL CLASSIFICATION

The gallbladders were classified histologically as outlined in Materials and Methods. Six (6%) gallbladders were normal, 54 (51%) had chronic mild cholecystitis

29 (28%) had chronic advanced cholecystitis and 17 (16%) had acute cholecystitis. (Fig. 4)

Normal Gallbladder (n = 6)

The mucosal layer in the normal gallbladder was usually folded forming villi and was separated from the muscle layer by a very thin layer of areolar connective tissue (Fig. 5).

The muscle layer was thin being composed of 2 - 4 muscle bundles which were multi-directional. There was elastic tissue interspersed between these muscle bundles. At the interface between the muscle and areolar layers there were usually scattered inflammatory cells which were mainly lymphocytes. The outer layer consisted of loose areolar connective tissue.

Chronic Mild Cholecystitis (CMC ; n = 54)

In this group there was mucosal hyperplasia with the mucosal layer being thrown into prominent mucosal folds which were polypoid, (Fig. 6). The mucosa was normal and there was no ulceration. The submucosa again was a thin layer of connective tissue which separated the mucosa from the muscle layer. In a small number of gallbladders there were mucosal glands scattered throughout the submucosa. In some cases it was difficult to differentiate these mucosal glands from Rokitansky-Aschoff sinuses.

FIGURE 4 : GALLBLADDER DISTRIBUTION

Histogram showing the distribution of gallbladders used in this study The greatest number were in the chronic mild cholecystitis (CMC) group, with decreasing numbers in the chronic advanced cholecystitis (CAC), acute cholecystitis (AC) and normal (Norm) groups.

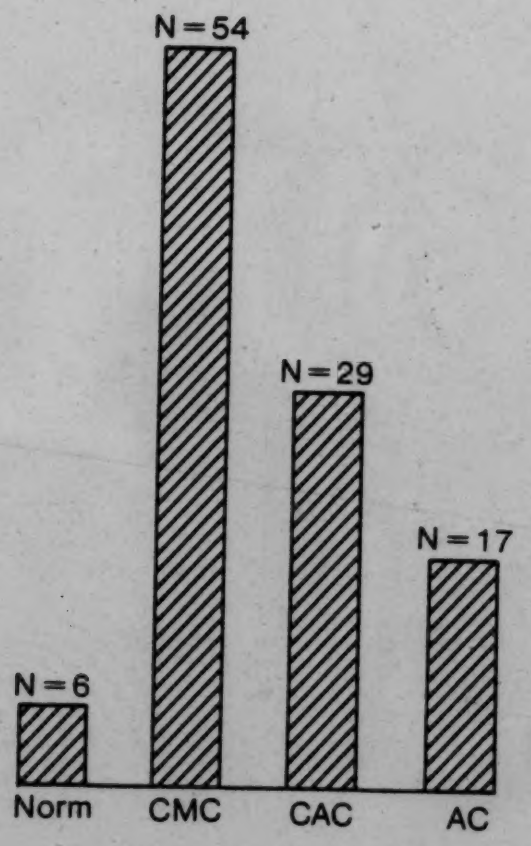

N=54
N=29
N=17
N=6
Norm
CMC
CAC
AC

FIGURE 5 : GALLBLADDER WALL

Photomicrographs of transverse sections through gallbladder wall (Haematoxylin and Eosin):

A. Normal gallbladder wall. Note muscle thickness and the mucosal folds (x50).

B. Chronic mild cholecystitis: this is an extreme example of the muscle hypertrophy noted in this condition (x50).

C&D. Examples of Rokitansky-Aschoff sinuses in chronic mild cholecystitis. The "sinus" (mucosal diverticulum) penetrates the entire muscle layer which is hypertrophied (x50).

E. Example of cholesterolosis showing cholesterol polyp (x50).

F. High power view of cholesterol polyp in E showing typical foam cells (x120).

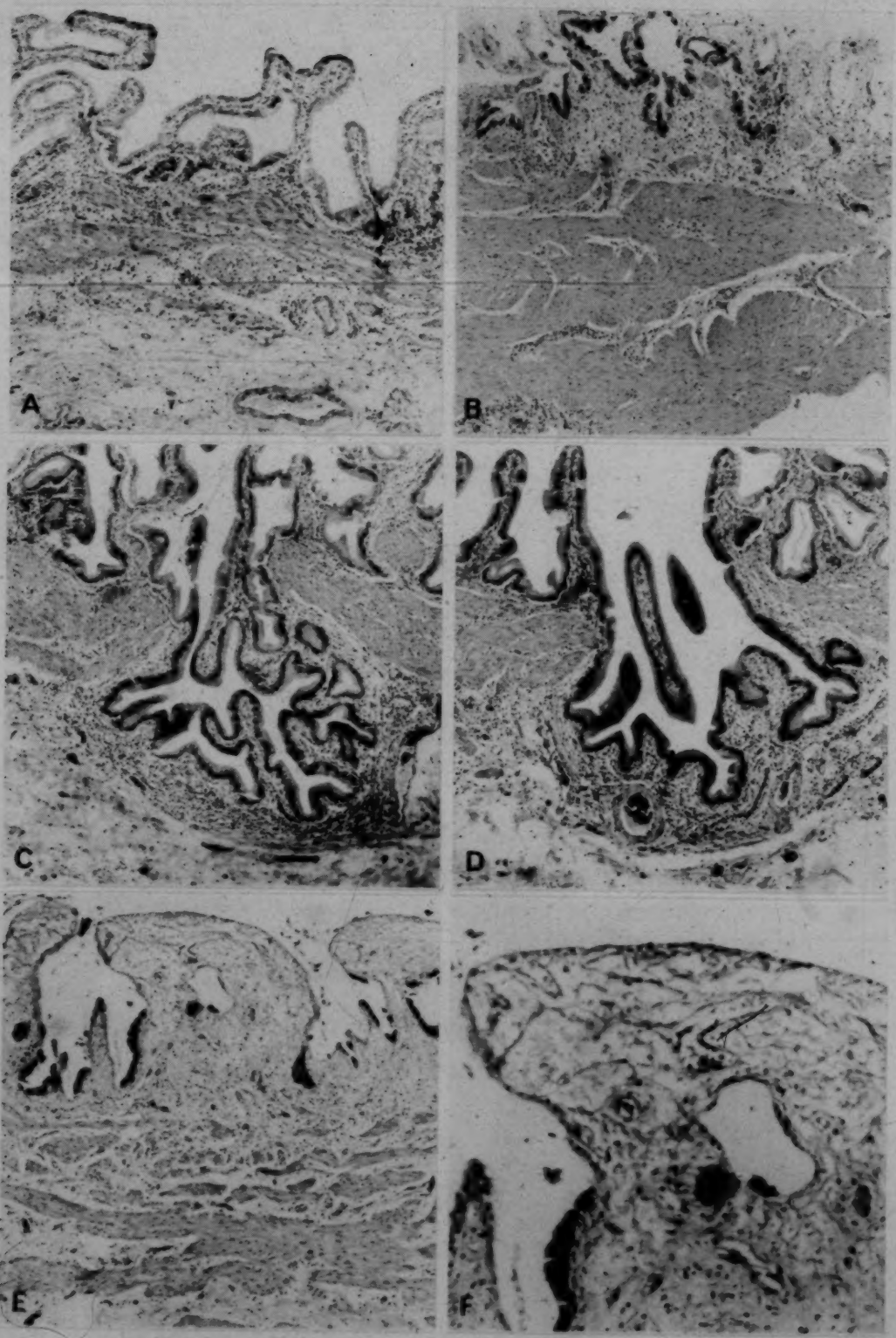
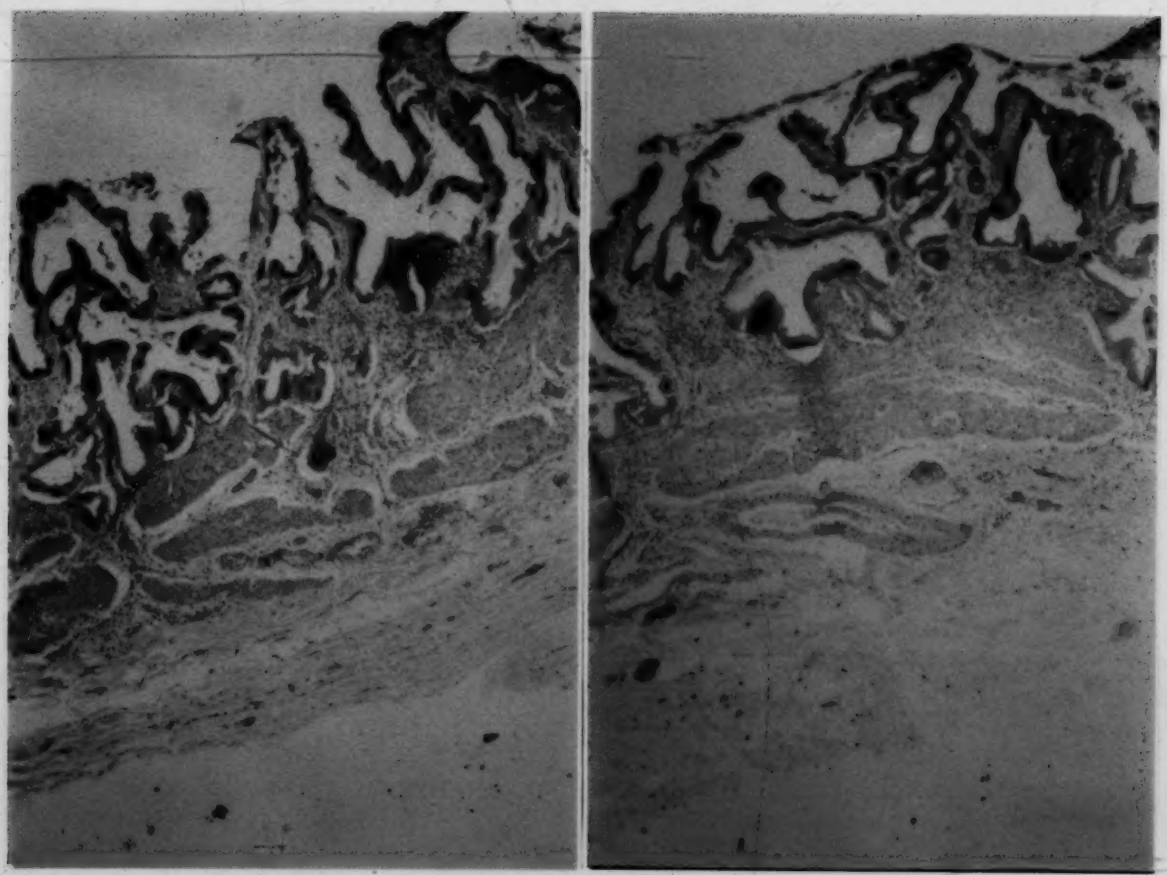

A
B
C
D
E
F

FIGURE 6 : CHRONIC MILD CHOLECYSTITIS

A & B Photomicrographs of transverse sections through gallbladder wall with chronic mild cholecystitis illustrating mucosal hyperplasia and muscle hypertrophy (Haematoxylin and Eosin, x50).



A B

Rokitansky-Aschoff sinuses were present in 64% of gallbladders in this group. In approximately half of these the sinuses were extensive and penetrated the full muscle thickness, (Fig. 5).

A most significant feature in this group was the marked muscle hypertrophy, the muscle bundles were hypertrophied and there was much less elastic tissue interspersed between the muscle bundles. As in the normal gallbladders there were occasional inflammatory cells, again mainly lymphocytes, at the interface of the muscle and areolar layers, with occasional follicular formation.

The areolar layer consisted of loose connective tissue and occasionally contained scattered lymphocytes.

Cholesterolosis which appeared as large cholesterol filled cells, macrophages or histiocytes, and usually referred to as foam cells, were noted in 11 gallbladders. The foam cells were scattered throughout the lamina propria and in some cases were seen as cholesterol polyps, i.e. large aggregates of foam cells covered by an intact epithelium (Fig. 5,8).

Acalculous Cholecystitis (n = 5)

These five gallbladders were classified as chronic mild cholecystitis. All five had varying degrees of mucosal polyposis and all had cholesterolosis (Fig. 5,7,8). Three of these gallbladders had Rokitansky-Aschoff sinuses, in two of which they extended throughout the full muscle thickness.

FIGURE 7 : GROSS CHOLESTEROLOSIS

Photograph of gallbladder wall with severe polypoid cholesterolosis.

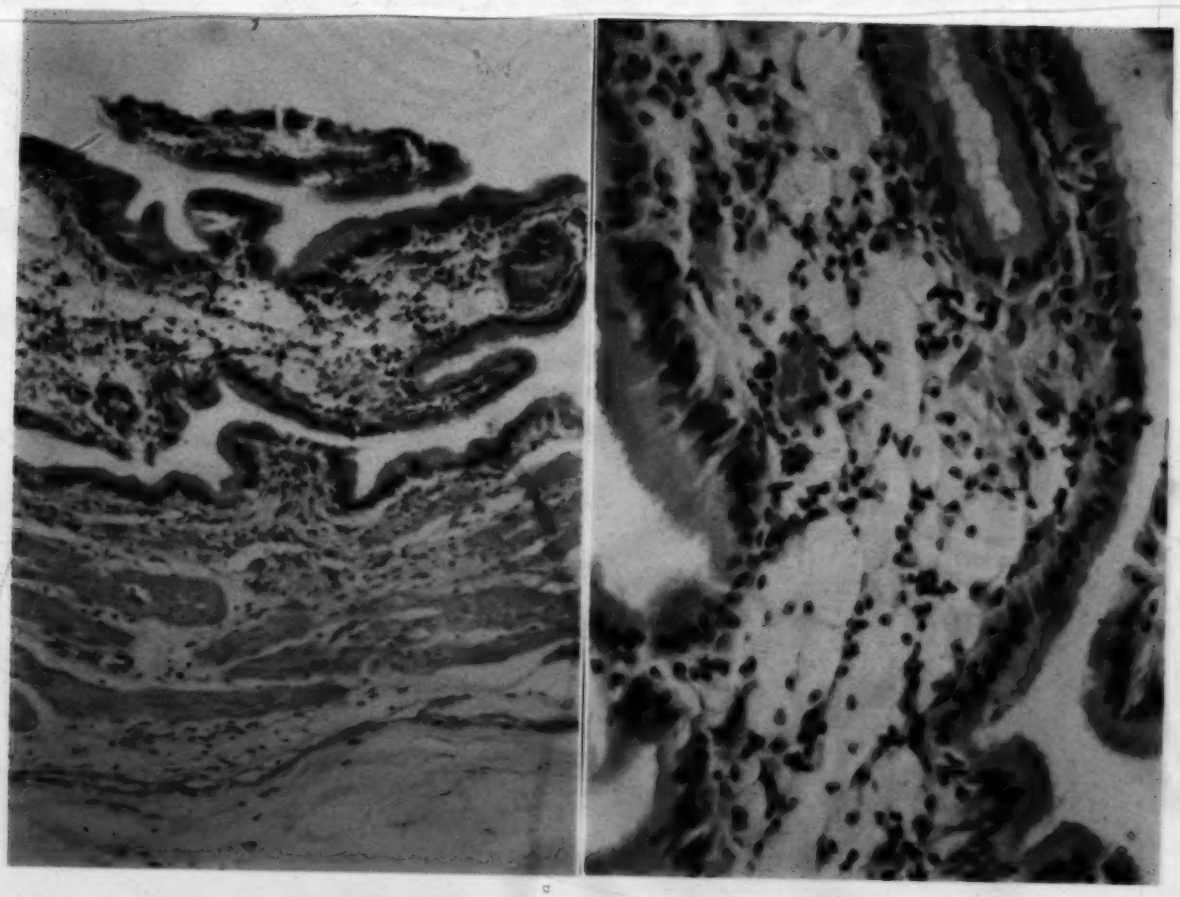
Scale

0 3 cms

FIGURE 8 : CHOLESTEROLOSIS

A. Photomicrograph of transverse section (haematoxylin and Eosin) of gallbladder wall with cholesterolosis showing cholesterol polyp (x50).

B. A higher power view of cholesterol polyp in A showing a collection of typical foam cells surrounded by intact epithelium (x120).



A B

Muscle hypertrophy was also evident in all these gallbladders. The degree of inflammatory infiltration was minimal similar to the other gallbladders in the chronic mild cholecystitis group.

Chronic Advanced Cholecystitis (CAC; n = 29)

In this group the polypoid mucosa of the chronic mild cholecystitis was replaced by atrophic mucosa which was flat and in some cases the epithelium was completely replaced by fibrous tissue. There were varying degrees of inflammatory infiltration of the mucosal layer of inflammatory cells, mainly lymphocytes, with occasional lymphoid mucosal follicles.

Muscle hypertrophy was evident in all specimens of this group. However, there was evidence of muscle destruction and replacement by fibrous tissue, in some places the entire muscle thickness was replaced by fibrous tissue. There was also infiltration of the muscle layer by increasing numbers of lymphocytes and plasma cells with the occasional lymphoid follicle formation.

The areolar layer was also infiltrated by inflammatory cells and in some places the loose connective tissue was completely replaced by fibrous tissue.

Acute cholecystitis (n = 17)

In all the specimens in this group the findings in the chronic advanced disease were present but were more extensive.

Acute inflammation was evident in all layers. In the mucosal layer there was ulceration and infiltration by polymorphonuclear leucocytes. There was also extravasation of red-blood cells.

In the muscle layer there was more extensive replacement of the muscle by fibrous tissue and infiltration by polymorphonuclear leucocytes was evident. In the areolar layer the connective tissue was extensively, and in some cases completely, replaced by fibrous tissue.

Mucocoele (Hydrops)

Here the outstanding feature was extreme thinning of all layers. The mucosal layer usually consisted of an intact epithelium which was flattened and atrophic. The muscle layer was also very thin as was the areolar. There was sparse inflammatory infiltration of all layers.

MUSCLE THICKNESS

(Fig. 9)

Normal Gallbladder

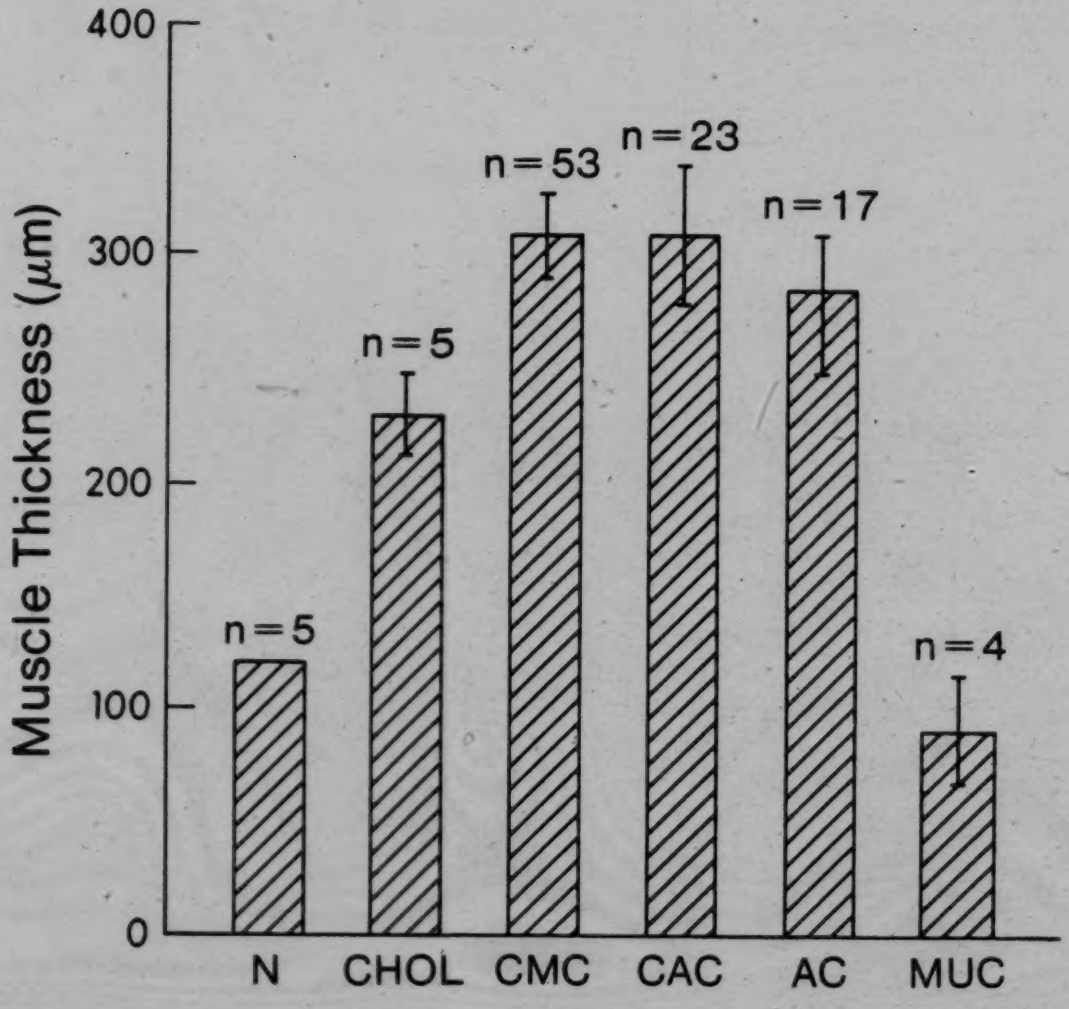
In the 6 normal gallbladders the muscle thickness fell within a narrow range of 116 - 123 μm: the mean thickness was 119 ± 3 μm which was significantly less than that in the diseased groups, calculous and acalculous.

Cholesterolosis

In this group the smallest muscle thickness was 182 μm, i.e., 50% thicker than normal gallbladder.

FIGURE 9 : GALLBLADDER WALL MUSCLE THICKNESS

Histogram showing muscle thickness in normal (N) gallbladders and those with cholesterolosis (Chol), chronic mild cholecystitis (CMC), chronic advanced cholecystitis (CAC), acute cholecystitis (AC) and mucocoele (MUC). Measurements excluded the intermuscle-bundle connective tissue. Bars represent the standard deviation.


Muscle Thickness (μm)
400
300
200
100
0
n=5
n=5
n=53
n=23
n=17
n=4
N
CHOL
CMC
CAC
AC
MUC

The mean thickness of the muscle in this group was 230 ± 55 µm.

Chronic Mild Cholecystitis

The muscle thickness in this group was very variable from the thinnest of 170 um to the thickness of 642 um with a mean thickness of 309 ± 36 um. In this group there was no muscle destruction (Fig. 4,5).

Chronic Advanced Cholecystitis

The mean muscle thickness in this group was the same as in the chronic mild group, i.e. 309 ± 61 µm. In one specimen the muscle measured 153 µm but this was due to areas of complete muscle destruction and replacement by fibrous tissue. In areas where there was no muscle damage the thickness was 3 - 4 times normal. The greatest muscle thickness recorded in this group was 582 µm, i.e. almost 5 times normal.

Acute Cholesystitis

In this group also there was a wide range of muscle thickness. Again there were some specimens with very thick mean muscle thickness which was due to areas of complete destruction and replacement by fibrous tissue. The mean thickness in this group was 285 ± 79 µm and with a range of 140 to 510 µm.

Mucocele (Hydrops)

The mean thickness in this group was 92 ± 24 μm which was thinner than normal. These gallbladders (5) were very distended at operation and were extremely thin-walled. There was no fibrosis in the walls of these organs.

MOTILITY STUDIES

Organ bath studies were carried out on 352 gallbladder strips. The effects of various agonist and antagonists on base tension, spontaneous activity, and contractility were studied.

Base Tension

Preliminary experiments were carried out to establish the ideal base tension for these strips, i.e. the tension level at which repeated stimulation by the same concentration of the same agonist yielded consistent responses. Strips from the different disease groups were assessed as tensions varying from 0.25 - 2 g. and stimulated repeatedly by acetylcholine (1mM). Consistent responses were seen at all tensions; however, some strips were unable to maintain base tensions of 1.5 g and greater and therefore a base tension of 0.5 g was used throughout the study.

In the studies with guinea pig gallbladder strips, the strips were set at a base tensions of 1 g as this is the tension reported in the literature to be most suitable.

In the human tissues incubation with the prostaglandin synthetase inhibitor indomethacin, caused a slight depression in base tension in a small number of strips. Incubation with the histamine H_1 receptor antagonist diphenhydramine (DPH) caused a very slight decrease in base tension in occasional gallbladder strips, while other antagonists such as the antimuscarinic agent atropine and the β-blocker propranolol caused no change in base tension.

In guinea pig gallbladder strips incubation with indomethacin (10 uM) caused a decrease in base tension from 40 - 70% while incubation with the diphenhydramine (10 uM) caused a lesser decrease in base tension of 15 - 20%. Other antagonists had no effect on base tension in these tissues.

Spontaneous Activity

Following suspension of the strips in oxygenated Krebs' solution the tension in gallbladder strips usually dropped over the next 5 - 10 minutes after which the tone usually increased. Concurrent with this increase in tone most strips developed spontaneous rhythmical contractions which varied in amplitude and frequency from gallbladder to gallbladder and indeed from strip to strip in the same gallbladder. Spontaneous activity that had regular rhythmic frequency and an amplitude greater than 3 mm was classified as good and that which was irregular or of low amplitude was classified as fair. Spontaneous activity was good in 239 (70%), fair in 46 (13%) and did not develop in 59 (17%)

gallbladder strips. Figure 10 shows good examples of spontaneous activity in gallbladder body strips.

This spontaneous activity, once established, persisted for several hours after which it gradually decreased in amplitude and frequency. The spontaneous activity was affected to a variable degree by contractile responses to agonists. In most strips it continued superimposed on small responses but its amplitude decreased with increasing contractile responses and it was abolished by maximal contractions to cholecystokinin-octapeptide and acetylcholine.

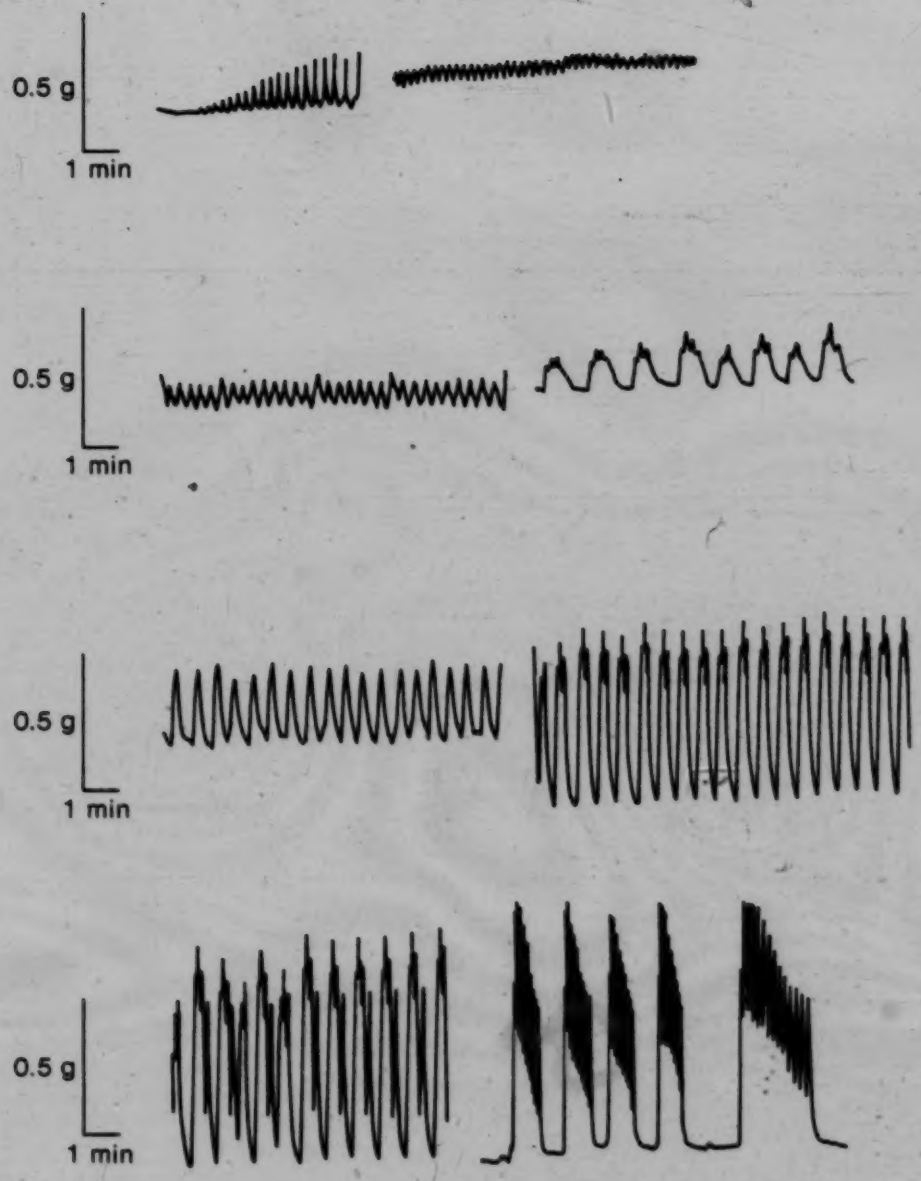
In guinea pig gallbladder strips the spontaneous activity was considerably more marked than in the human strips being present in all the strips studied and being of much greater amplitude so that at times it obscured the contractile responses to cholecystokinin-octapeptide.

When human strips were incubated with indomethacin (10 μM) there was an obvious suppression of the amplitude but not the frequency of spontaneous activity. There was a slight, but noticeable, decrease in spontaneous activity in human strips when incubated with the histamine H_1 antagonist diphenhydramine 10 μM.

In the guinea pig strips spontaneous activity, no matter how marked, was quickly abolished by indomethacin (10 μM) and was decreased significantly by diphenhydramine (μM).

FIGURE 10 : SPONTANEOUS ACTIVITY

Examples of good spontaneous activity in gallbladder body strips, example at top left shows development of spontaneous activity in strip after suspension in organ-bath.


0.5 g
1 min
0.5 g
1 min
0.5 g
1 min
0.5 g
1 min

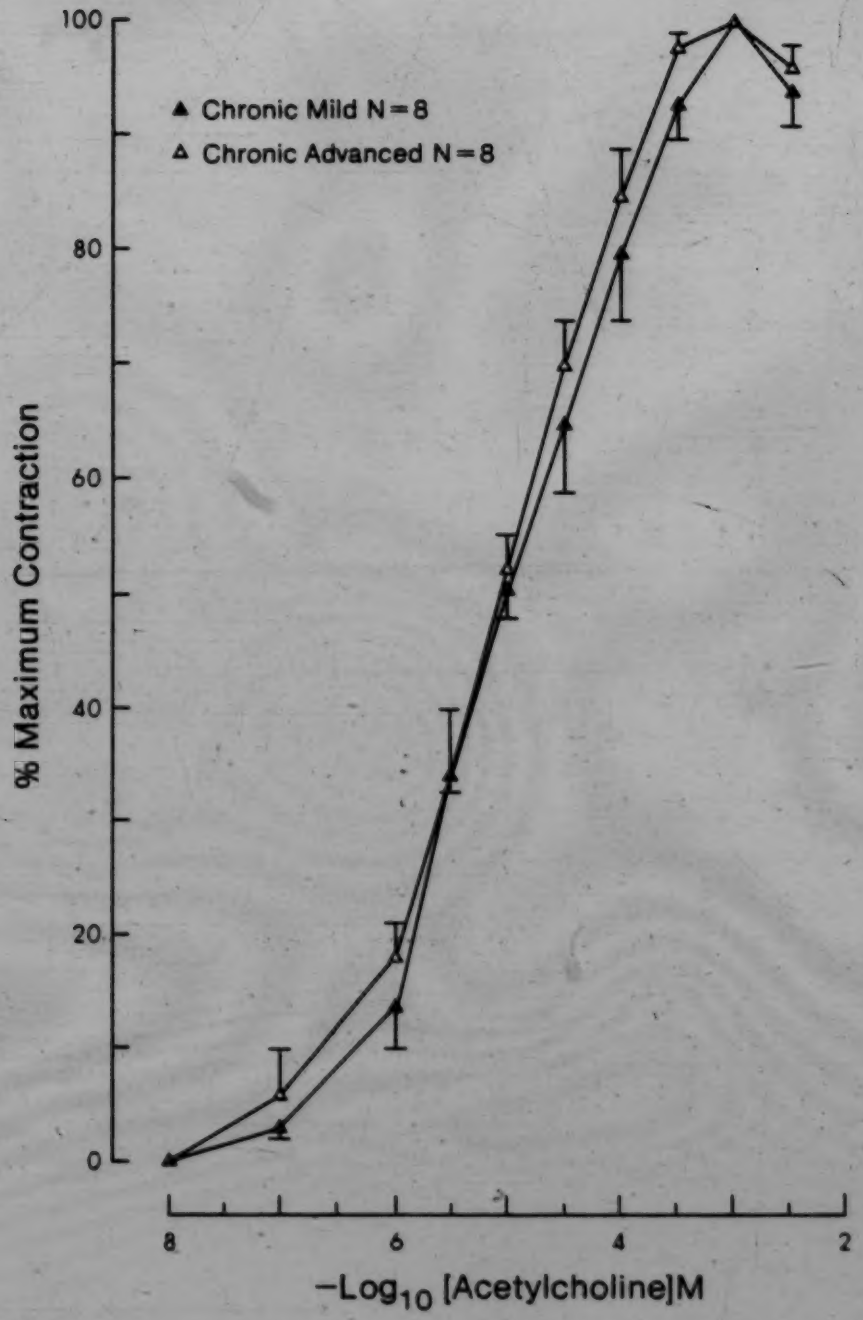
ACETYLCHOLINE

In this and in the following sections,except where otherwise stated, gallbladder strips refers to human gallbladder strips. Acetylcholine produced concentration-dependent contractions in all gallbladder strips tested. The onset of action following application of acetylcholine was rapid, being approx. 5 - 10 s., and the maximal contractile effect was reached between 60 and 80 s. The time required to reach maximal response was the same for small and large acetylcholine concentrations. The tone returned to the baseline in approximately 6 min. and this was accelerated by washing with fresh Krebs' solution.

Full concentration-response curves were constructed for strips from 16 gallbladders, 8 with chronic mild cholecystitis and 8 with chronic advanced cholecystitis (Fig. 11). The mean maximum contractile response in the chronic mild group was 1.75 g while that in the chronic advanced group was 1.655 g. - the difference was not statistically significant. The ED_{50} (i.e. the concentration required to cause half the maximal response) was calculated for the chronic mild and chronic advanced groups. The sensitivity (ED_{50}; 95% confidence limits) for the chronic mild cholecystitis group was 11; 9 - 11 µM, while that for the chronic advanced group was 10; 6-25 µM. Here again the difference was not statistically significant.

FIGURE 11 : CONCENTRATION-RESPONSE CURVE FOR ACETYLCHOLINE

Concentration-response curves for isolated strips of human gallbladder with chronic mild cholecystitis and chronic advanced cholecystitis to acetylcholine. Contractile responses are expressed as a percentage of the maximal response of each strip. Points represent the mean ± SEM.


100
80
60
40
20
0
% Maximum Contraction
▲ Chronic Mild N=8
△ Chronic Advanced N=8
8
6
4
2
−Log10 [Acetylcholine]M

AGONISTS AND ANTAGONISTS OF ADRENERGIC RECEPTORS

Adrenaline, Noradrenaline

It was found during preliminary experiments that there was no difference in the effects, efficacy or potency, of noradrenaline and adrenaline on gallbladder strips. Therefore, only noradrenaline was used for the remainder of the study. In strips from 11 gallbladders studied noradrenaline caused relaxation, and in 2 of these a biphasic effect was seen, low concentrations causing slight contractile responses and the higher concentrations causing relaxation. Onset of action was 10 - 15 s. following drug administration and maximum relaxant effect was achieved slowly; maximum relaxant effect to 100 uM was reached in 6 - 8 min. (Fig. 12). Following this the return of the tension to baseline was very slow, taking up to 30 min. and seemingly unaffected by washing with Krebs' solution.

The maximal relaxant effect of 18.4 $\pm$ 6.0% reduction of base tone was caused by a concentration of 100 µM. The relaxant effect of noradrenaline was more apparent on strips previously contracted by sub-maximal concentrations of cholecystokinin octapeptide. However, it was not possible to demonstrate any relaxant effect on strips contracted by near maximal concentrations (100 µM) of this agent.

The concentration response curve to noradrenaline is shown in Fig. 13. The ED_{50} for noradrenaline in gallbladder strips was 19 $\pm$ 5 µM (Fig. 13).

FIGURE 12 : NORADRENALINE AND PHENYLEPHRINE

A_1 Tracing showing the relaxant effect of noradrenaline (1mM) (↑) on resting tension in gallbladder strip.

A_2 Tracing showing the relaxant effect of noradrenaline (1 mM) (↑) on a cholecystokinin (10 nM) (↑) contracted strip.

B. Tracings showing the contractile effect of phenylephrine (1mM) (↑) on gallbladder strips.

▲ Indicates washing of strips with fresh Kreb's solution.

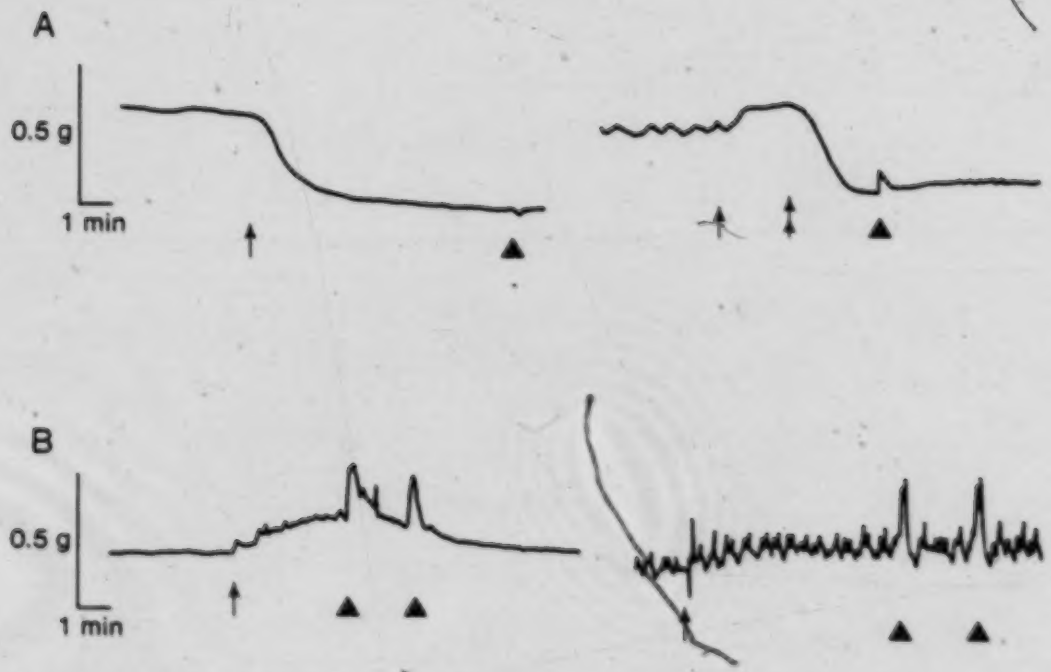
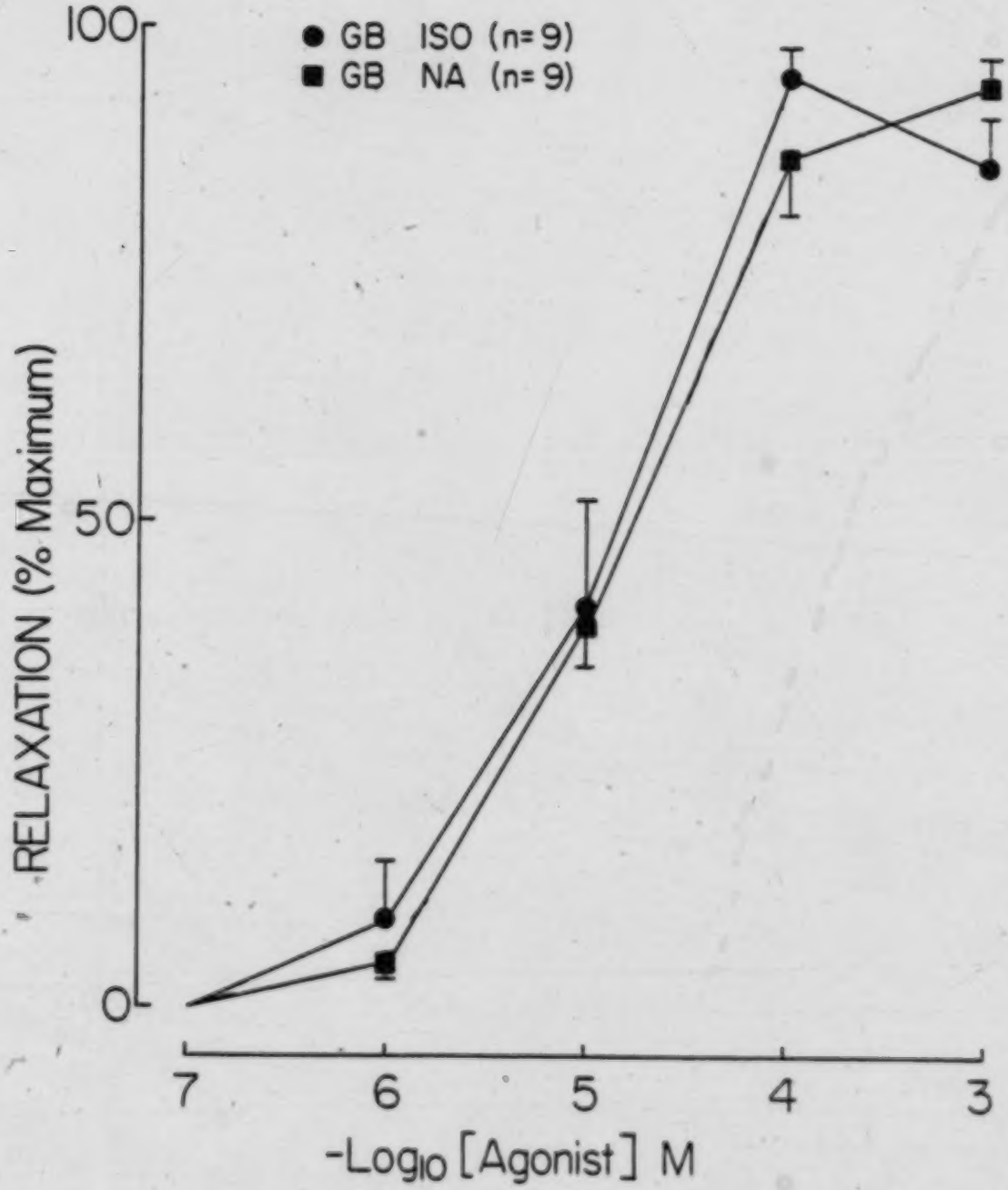

A
0.5 g
1 min
B
0.5 g
1 min

FIGURE 13 : CONCENTRATION-RESPONSE CURVES FOR NORADRENALINE AND ISOPRENALINE.

Concentration-response curves for isolated strips of human gallbladder to isoprenaline (ISO) and noradrenaline (INA). Relaxant responses are expressed as a percentage of the maximal response of each strip. Points represent the mean ± SEM.


100
50
0
● GB ISO (n=9)
■ GB NA (n=9)
RELAXATION (% Maximum)
7
6
5
4
3
-Log10 [Agonist] M

Isoprenaline

Isoprenaline caused concentration-related relaxation in 9 of 10 gallbladders studied. No contractile effect was seen. Like noradrenaline the onset of action was 10 - 15 s., maximum relaxant effect was seen in 6 - 8 min. and the time to return to base was up to 30 min. Relaxation was seen with concentrations of 1 nM and maximal relaxant effect was with 1 μM. The maximal relaxations produced by isoprenaline (mean 30 ± 5.6% reduction in base tension) were greater than those produced by noradrenaline (18.4 ± 6%). As with noradrenaline the relaxant effect with isoprenaline was more apparent on pre-contracted strips, relaxing strips contracted with cholecystokinin-octapeptide (1pM - 10 nM). However, isoprenaline had no relaxant effect on CCK-OP maximally contracted strips.

Gallbladder sensitivity to isoprenaline, ED_{50} - 15; 9 - 23 μM, did not differ significantly from that to noradrenaline, ED_{50} 19 ± 5 μM, (Fig. 13).

Beta-Blocking Agents

The relaxant effect of isoprenaline and noradrenaline on both resting and CCK-OP induced tension were antagonised by propranolol (100 μM - 1 mM). In 8 of the 11 gallbladder bodies stimulated with noradrenaline, propranolol unmasked a small contractile response. No contractile response were seen with isoprenaline in this way. The maximum contractile effect of noradrenaline (10 uM) in the presence

of propranolol was approximately 5% of the maximal CCK-OP response. The β_1 selective antagonist, practolol, (1 uM - 1 mM) had no effect of noradrenaline and isoprenaline-produced relaxations.

Phenylephrine

The effect of the α-receptor agonist phenylephrine (1 μM - 3 mM) was studied in 22 gallbladder body strips. Contractile responses were seen in strips from only 2 gallbladders, (Fig. 12). The maximal contractile effect, however, was less than 5% of the maximal CCK-OP effect and were abolished by the α-receptor antagonist phentolamine (100 uM).

FIELD STIMULATION

The effects of field-stimulation were studied on 22 gallbladders, 16 with chronic mild cholecystitis, 5 with chronic advanced cholecystitis and one normal. Gallbladder strips were suspended in the organ bath between platinum electrodes. A current of 100 volts was used throughout the experiment. Maximal effects in different tissues were produced by widely different frequencies and durations of stimulation.

Stimulation in the absence of antagonists always produced contractile responses (Fig. 14). Maximal contractile responses were small, being less than 30% of the maximum acetylcholine response. Contractile responses

FIGURE 14 : EFFECTS OF FIELD-STIMULATION

A. Tracing of contractile effect of field stimulation ⟷ on gallbladder strip.

b. Tracing of the relaxant effect of field-stimulation ⟷ on gallbladder body in the presence of atropine.

A
0.5 g
1 min
B
0.5 g
1 min

were antagonised by the muscarinic antagonist, atropine, in a concentration of 100 μM. Stimulation of strips in the presence of a higher concentration of atropine (1 mM) caused relaxant responses in strips from 5 gallbladders including one normal (Fig. 14). In this situation maximal reduction of base tension of 40 - 50% was seen in these strips, i.e. a relaxant response greater than that produced by isoprenaline or noradrenaline. Stimulation in the presence of both atropine (1 mM) and propranolol (100 uM) produced very small contractile responses in strips from 3 gallbladders; maximal acetylcholine stimulation (1 mM) caused no response in these strips, indicating that the contractile response was caused by adrenergic nerve stimulation. No relaxant effects were seen in response to field-stimulation in the presence of atropine and propranolol.

HISTAMINE

The effects of histamine stimulation was studied on 27 gallbladders and in concentrations of 10μM to 3 mM and produced concentration-dependent contractions in all strips. Contractile responses to histamine were rapid in onset (5 - 10 seconds), reached a maximum quickly (60 - 90 seconds), and returned to baseline gradually (approximately 10 minutes from maximal contraction).

A comparison of concentration-response curves for histamine in strips from gallbladders in each of the

diseased groups indicated significant differences in the sensitivity of histamine (Fig. 15). There was a significant correlation (P 0.01) between the degree of cholecystitis (histology score) and the sensitivity of the muscle strips to histamine (Fig. 16). ED_{50} values for the different groups are shown in table 2.

Histamine Receptor Characterization

Diphenhydramine (10 uM), an H_1 receptor antagonist, significantly antagonized the contractile effect of histamine (Fig. 17). A higher concentration of diphenhydramine (100 uM), tested in a group of 13 gallbladders, reduced or abolished the contractile response and unmasked a small relaxant response in 4 (Fig. 17). Cimetidine (100 uM), an H_2 receptor antagonist, did not affect the sensitivity or the maximal response of strips from 9 gallbladders to the contractile effects of histamine (Fig. 17). The H_1 receptor antagonist 2-pyridylethylamine (1 uM to 3 mM) produced contractile responses of gallbladder but was significantly less potent (Fig. 18) and elicited a smaller maximal contraction (61% of histamine maximum) than did histamine. The H_2 receptor antagonist 4-methylhistamine, also produced concentration-dependent contractile responses (Fig. 18). However, high concentrations produced only small responses and a maximum was not reached. The contractile responses to both 2-pyridylethylamine

FIGURE 15 : CONCENTRATION-RESPONSE CURVE FOR HISTAMINE

Concentration-response curves for isolated strips of human gallbladder to histamine. Contractile responses are expressed as a percentage of the maximal response of each strip. Curves are shown for strips which were graded histologically as normal and as having chronic mild cholecystitis (CMD), chronic advanced cholecystitis (CAD) or acute cholecystitis (Acute). Points represent the mean responses ± SEM.

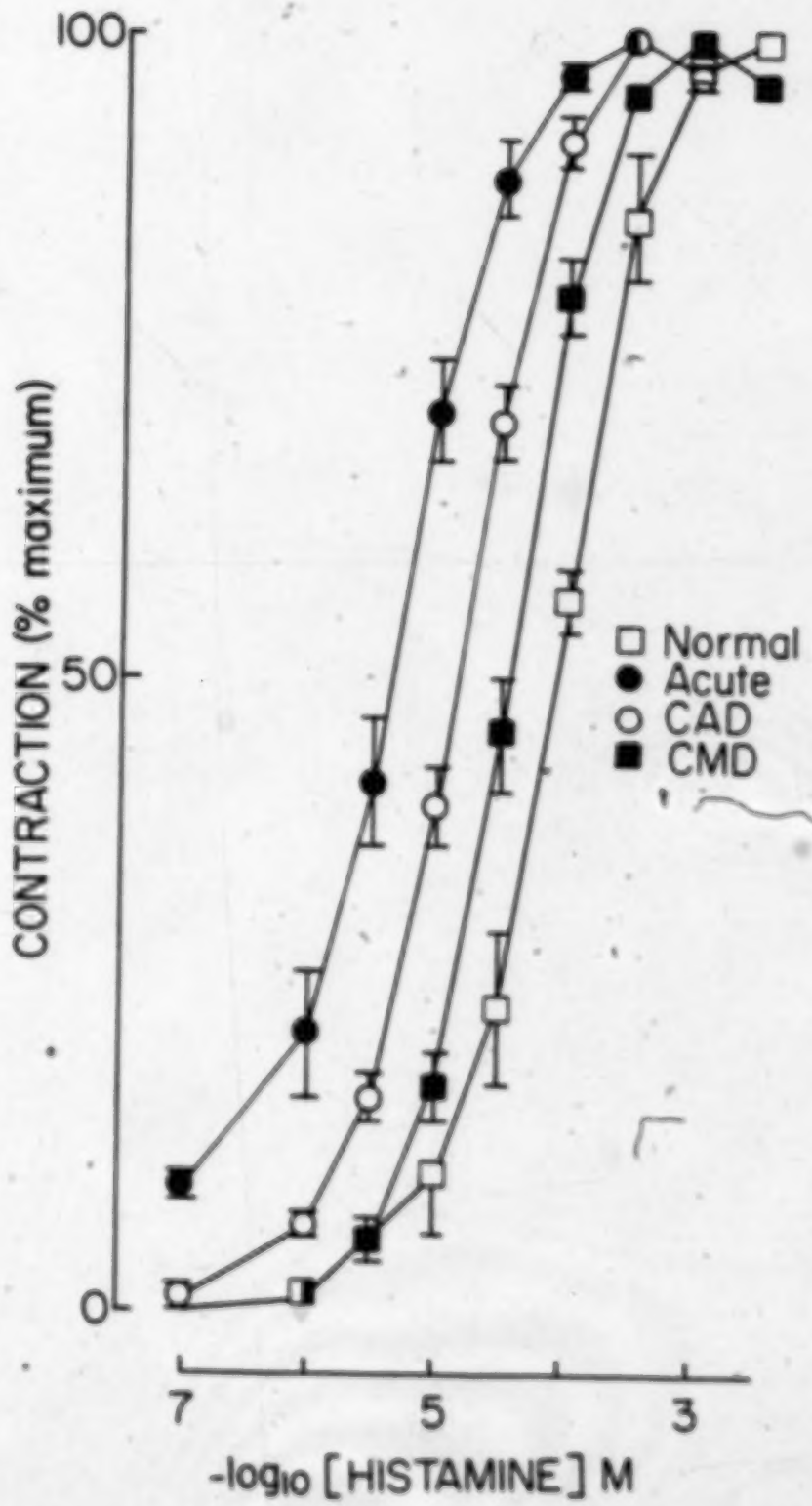

100
50
0
CONTRACTION (% maximum)
Normal
Acute
CAD
CMD
7
5
3
-log10 [HISTAMINE] M

FIGURE 16 : RELATIONSHIP BETWEEN HISTAMINE SENSITIVITY AND DEGREE OF GALLBLADDER INFLAMMATION

Relationship between the sensitivity of human gallbladder body strips to histamine (assessed by calculation of the ED_{50} from individual concentration-response curves) and the grade of disease (histology score) assessed by histological examination of adjacent strips from each gallbladder. Linear regression analysis revealed a significant correlation (r=0.989).

C Chronic mild cholecystitis

Ch Chronic advanced cholecystitis

A Acute cholecystitis

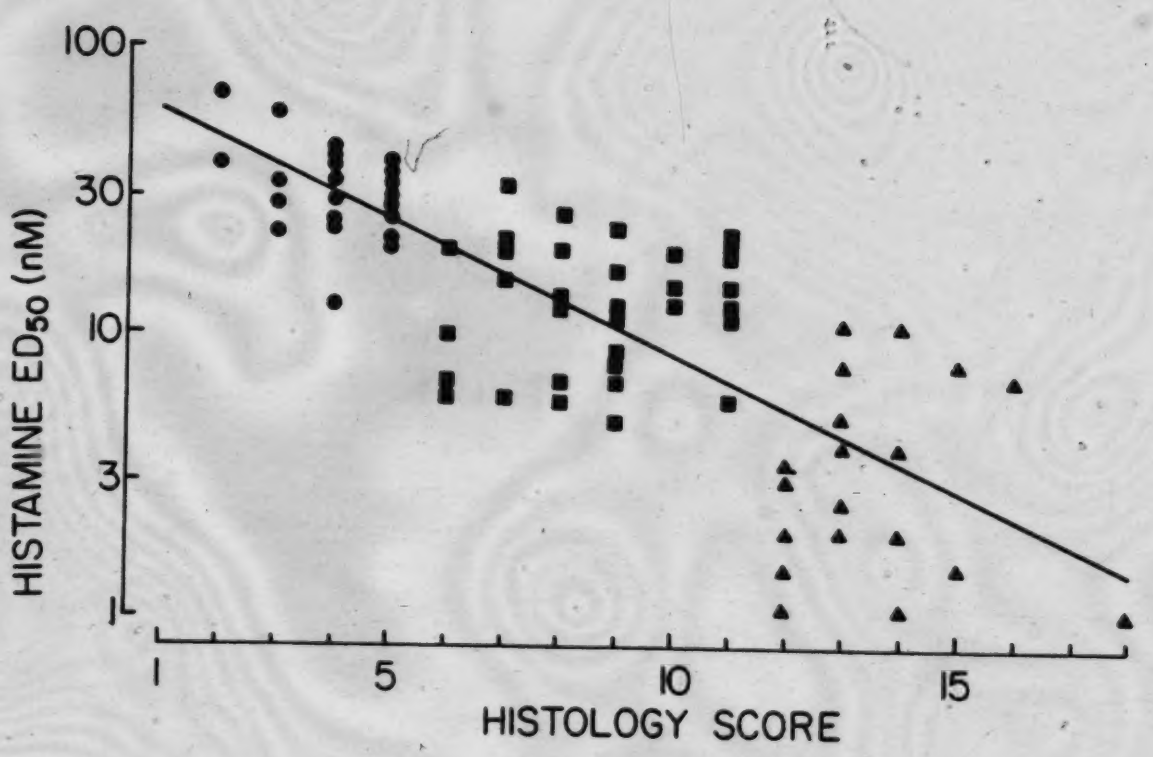

100
30
10
3
1
HISTAMINE ED50 (nM)
1
5
10
15
HISTOLOGY SCORE

TABLE 2 : Gallbladder Sensitivity to Histamine in normal and diseased states.

GROUP	ED_{50} (95% CONFIDENCE LIMITS) µM.
NORMAL	90 (31 - 135)
CMC	32.4 (28.8 - 37.4)
CAC	12.5 (10.0 - 15.9)
AC	3.0 (2.0 - 4.6)

CMC Chronic Mild Cholecystitis

CAC Chronic Advanced Cholecystitis

AC Acute Cholecystitis

FIGURE 17 : EFFECT OF HISTAMINE RECEPTOR ANTAGONISM

Concentration-response curves for isolated strips of human gallbladder body to histamine in normal Krebs and in the presence of cimetidine 100 μM and diphenhydramine 10 and 100 μM. Contractions are expressed as a percentage of the maximal control response to histamine on each strip. Approximately 30% of gallbladder strips displayed a small relaxant response (expressed as a percentage decrease in baseline tension) to histamine in the presence of diphenhydramine 100 M. Only data from gallbladders with chronic cholecystitis are included. Points represent mean ± SEM.

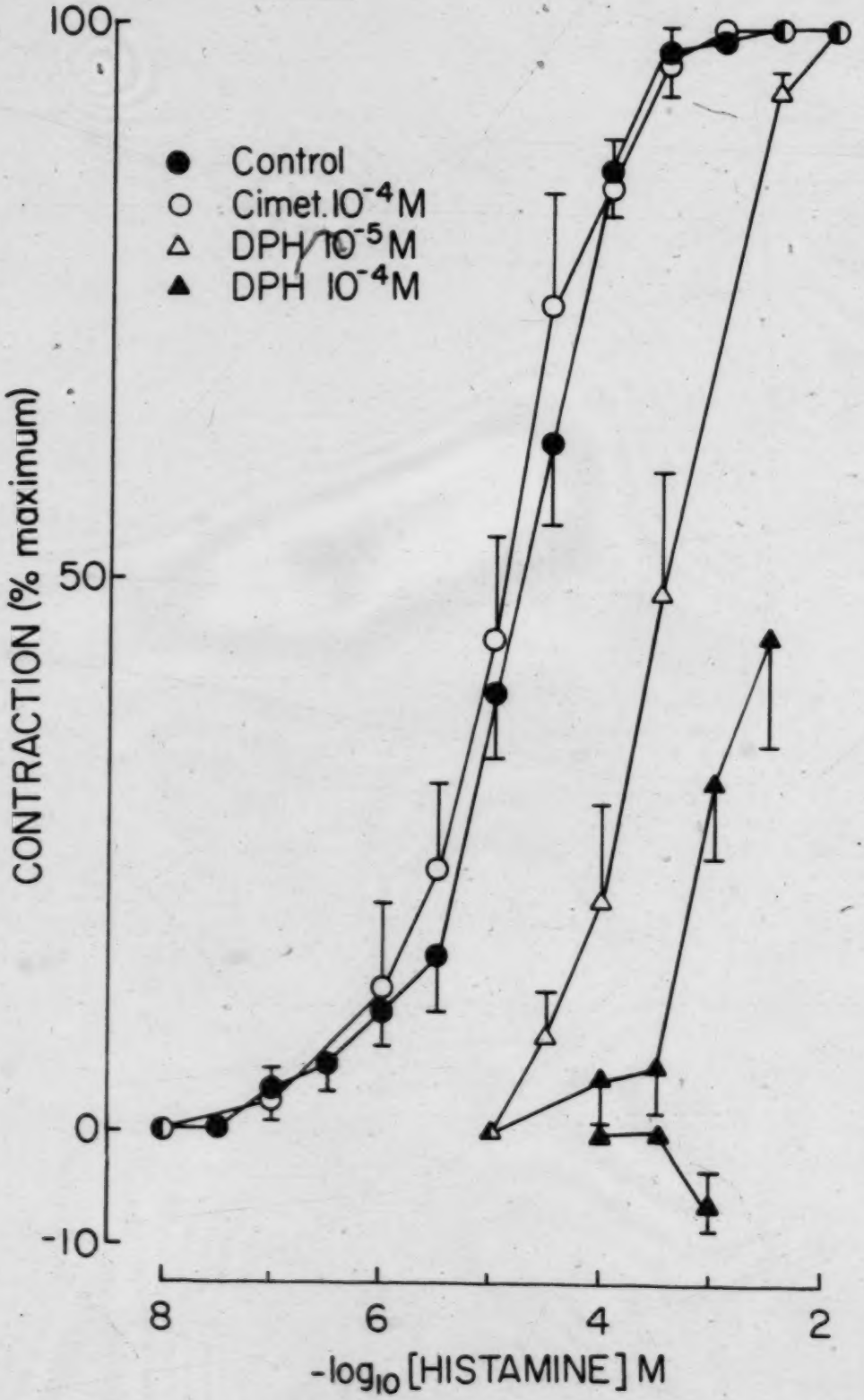
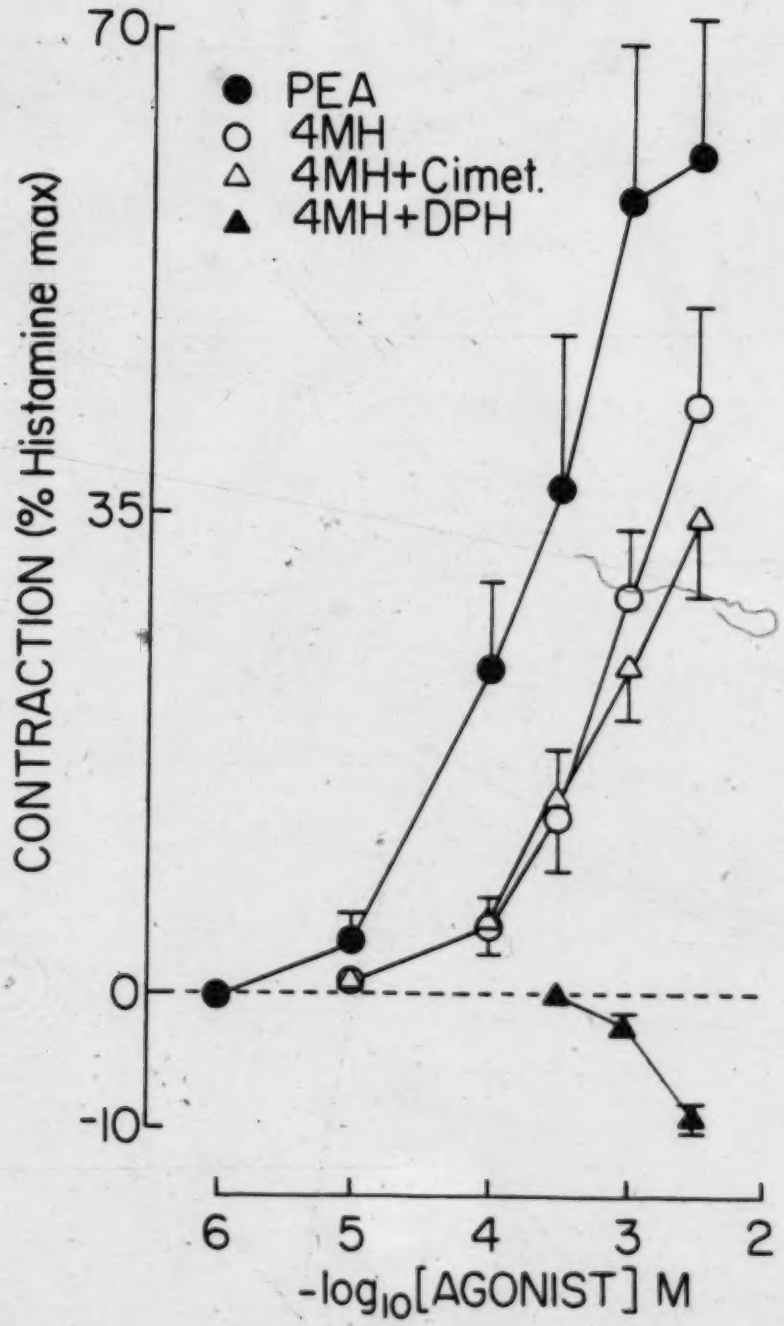

100
50
0
-10
CONTRACTION (% maximum)
Control
Cimet. 10^{-4} M
DPH 10^{-5} M
DPH 10^{-4} M
8
6
4
2
$-\log_{10}$ [HISTAMINE] M

FIGURE 18: CONCENTRATION-RESPONSE CURVES FOR 2-PYRIDYLETHYLAMINE AND 4-METHYLHISTAMINE

Concentration-response curves for isolated strips of human gallbladder body with chronic cholecystitis to 2-Pyridylethylamine (PEA) and 4-methylhistamine (4MH) in normal Krebs and in the presence of diphenhydramine 100 uM and cimetidine 10 uM. Contractile responses are expressed as a percentage of the maximal response to histamine in each strip. Relaxant responses were obtained in 43% of gallbladders to 4-methylhistamine in the presence of diphenhydramine 100 uM and are expressed as a percentage decrease in baseline tension. Points represent the mean $\pm$ SEM.


70
35
0
-10
CONTRACTION (% Histamine max)
PEA
4MH
4MH+Cimet.
4MH+DPH
6
5
4
3
2
-log10[AGONIST] M

and 4-methylhistamine were abolished by diphenhydramine (100 μM) and small relaxant responses to 4-methylhistamine were then observed in 6 of the 14 gallbladders studied (Fig. 18,19). Tolazoline, a specific H_2 receptor agonist caused contractile responses which were antagonised by diphenhydramine. No relaxant responses were seen with tolazoline in the presence of diphenhydramine. Impromidine (0.1 μM to 30 μM), a highly specific H_2 receptor agonist, failed to elicit a contractile or relaxant response in either the absence or presence of diphenhydramine (100 μM). In strips from 10 gallbladders in which the tone was raised by CCK-OP (10 nM) in the presence of diphenhydramine (100 μM), histamine (3 mM), 4-methylhistamine (3 mM) or impromidine (30 μM) did not elicit any relaxant responses.

Similar studies were carried out on a small number of guinea pig gallbladder strips. Stimulation with 4-methylhistamine and histamine in the presence of diphenhydramine had a potent relaxant effect. Contractile effects due to cholecystokinin octapeptide in the presence of diphenhydramine were antagonised, and in some cases completely reversed, by the addition of histamine or 4-methylhistamine.

HORMONAL STIMULATION

Cholecystokinin

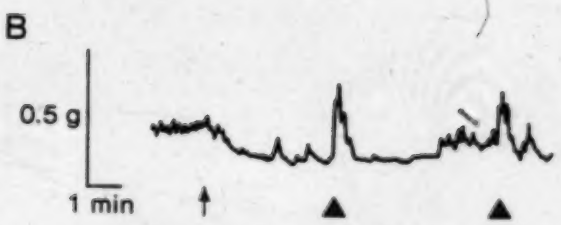
The effect of cholecystokinin was studied on 36 gallbladders. Two cholecystokinin preparations were

FIGURE 19 : EFFECTS OF HISTAMINE H_2 RECEPTOR STIMULATION

A & B : Tracings showing the relaxant effect of 4-methylhistamine (mM) (↑) on gallbladder strips in the presence of diphenhydramine (100 µM).

▲ Indicates washing of strip with fresh Krebs' solution.

A
0.5 g
1 min


B
0.5 g
1 min

used, a natural preparation which was quantified by bioassay, and a synthetic preparation consisting of the terminal (COOH) 8 amino acids (octapeptide). The characteristics of the responses (time of onset, time to peak and relaxation time) were similar for both preparations. As it was not possible to achieve a maximal response to the natural preparation only results obtained with the octapeptide preparation were used to calculate sensitivity and maximal responses.

Concentration-dependent contractions were caused by cholecystokinin-octapeptide (CCK-OP) in all gallbladder strips studied. The onset of action was more rapid with increasing concentrations being 36 $\pm$ 4 seconds and 11 $\pm$ 1 second at 1 pM and 1 µM concentrations respectively. The onset of action with any given concentration was similar in the different disease groups. The contractile response to CCK-OP was slow and the time taken to reach peak contractions increased with increasing concentrations so that in some strips the peak contractions to 10 µM CCK-OP was reached after 9 - 10 minutes. Figure 20 shows the time taken to reach maximum response for the different CCK-OP concentrations.

The most characteristic feature of the CCK-OP response was the long relaxation time which increased with increasing CCK-OP concentration. The time taken for strips to return to baseline tension following maximal CCK-OP stimulation was 44 $\pm$ 6 minutes. This return to base tension was seemingly unaffected by washing with fresh Krebs' solution (Fig. 21).

FIGURE 20 : TIME TO REACH MAXIMUM CONTRACTILE RESPONSE WITH CHOLECYSTOKININ-OCTAPEPTIDE

Diagram showing the time taken for increasing concentrations of CCK-OP to achieve maximal effect in strips from gallbladders with chronic mild cholecystitis (n = 38).

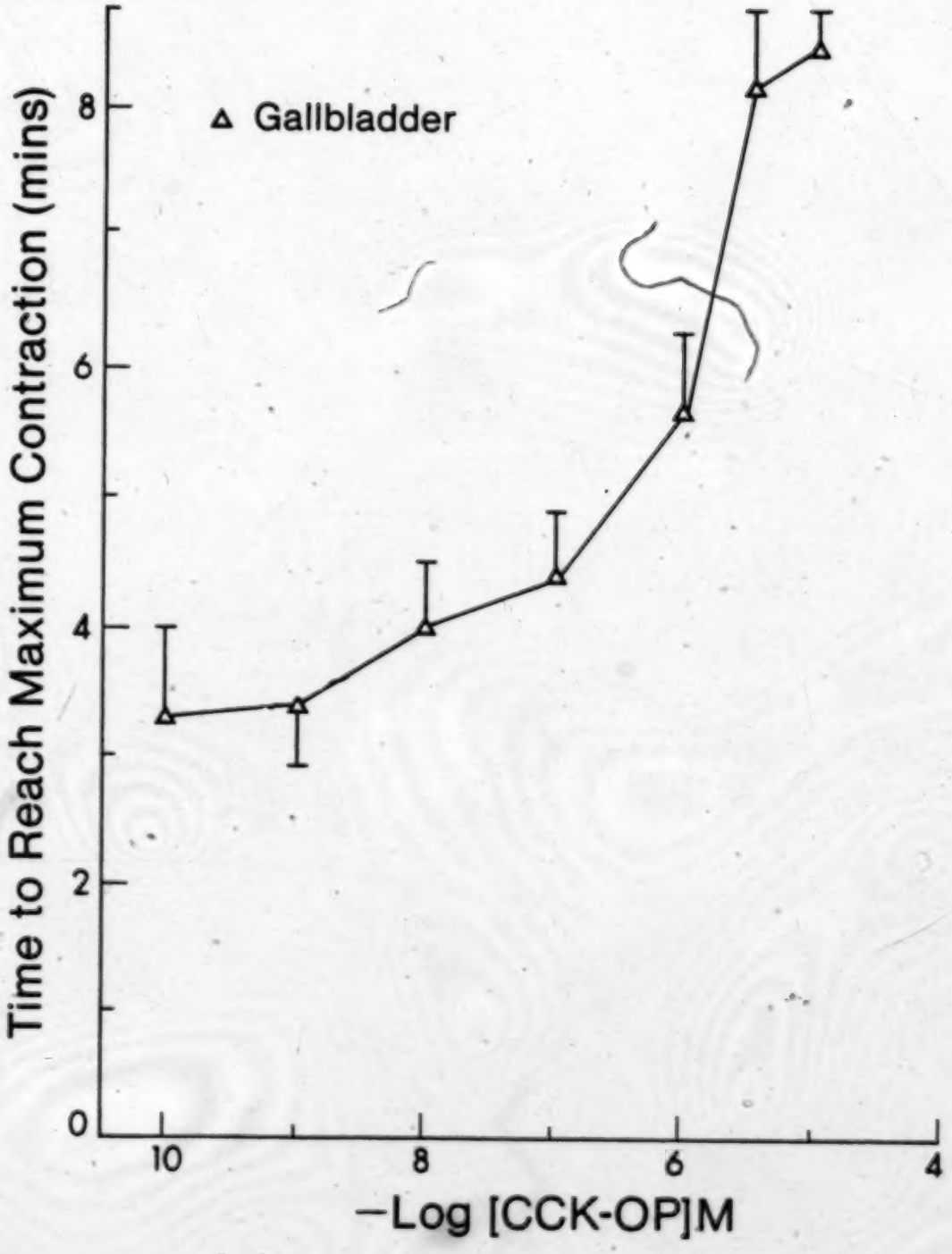

Time to Reach Maximum Contraction (mins)
0
2
4
6
8
Δ Gallbladder
10
8
6
4
−Log [CCK-OP]M

FIGURE 21 : RESPONSES TO CHOLECYSTOKININ-OCTAPEPTIDE CAERULEIN AND GASTRIN.

Tracings showing similarity of contractile responses by gallbladder to:-

A. CCK-OP (+) (10 μM)

B. Gastrin I (+) (10 μM)

C. Caerulein (+) 500 nM

▲ indicates washing of strip with fresh Krebs' solution.

A
1.0 g
5 min
B
1.0 g
5 min
C
1.0 g
5 min

The contractile effect of CCK-OP was unaltered by the antimuscarinic agent atropine (100 μM), the histamine H_1 and H_2 receptor antagonists diphenhydramine (100 μM) and cimetidine (100 μM) respectively, or the prostaglandin synthetase inhibitor indomethacin (10 μM). The CCK-OP effect was antagonised by N_2O_2-dibutyryl guanosine 3'-5' cyclic monophosphate (dbGMP) in concentrations of 1 - 300 μM. The antagonist effect was evident whether the dbGMP was added to the bath before, with or after the CCK-OP. The higher concentrations of dbGMP (300 μM) completely antagonised contractile responses to CCK-OP concentrations up to 1 nM, while responses to higher concentrations (3nM - 1 μM) were only partly antagonised, the maximal CCK-OP responses being only minimally affected. Whereas the CCK-OP contracted strip was unaffected by washing with fresh Krebs' solution, washing strips in which the CCK-OP contractile effect was antagonised by dbGMP caused the contractile effect to CCK-OP to be restored.

Full concentration-response curves were constructed for normal and diseased groups (Fig. 22,23). The data for the three gallbladders with acalculous cholecystitis was included in the chronic mild cholecystitis group and also considered separately. Estimation of the sensitivity (ED_{50}; 95% confidence limits) of gallbladder strips in the different groups revealed that no significant difference existed between normal gallbladders, those with acalculous

FIGURE 22 : CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE

Contractile responses were expressed as a percentage of the maximal response of each strip. Curves are shown for strips from normal gallbladders and those with chronic mild, chronic advanced, and acute cholecystitis. Points represent the mean responses ± SEM.

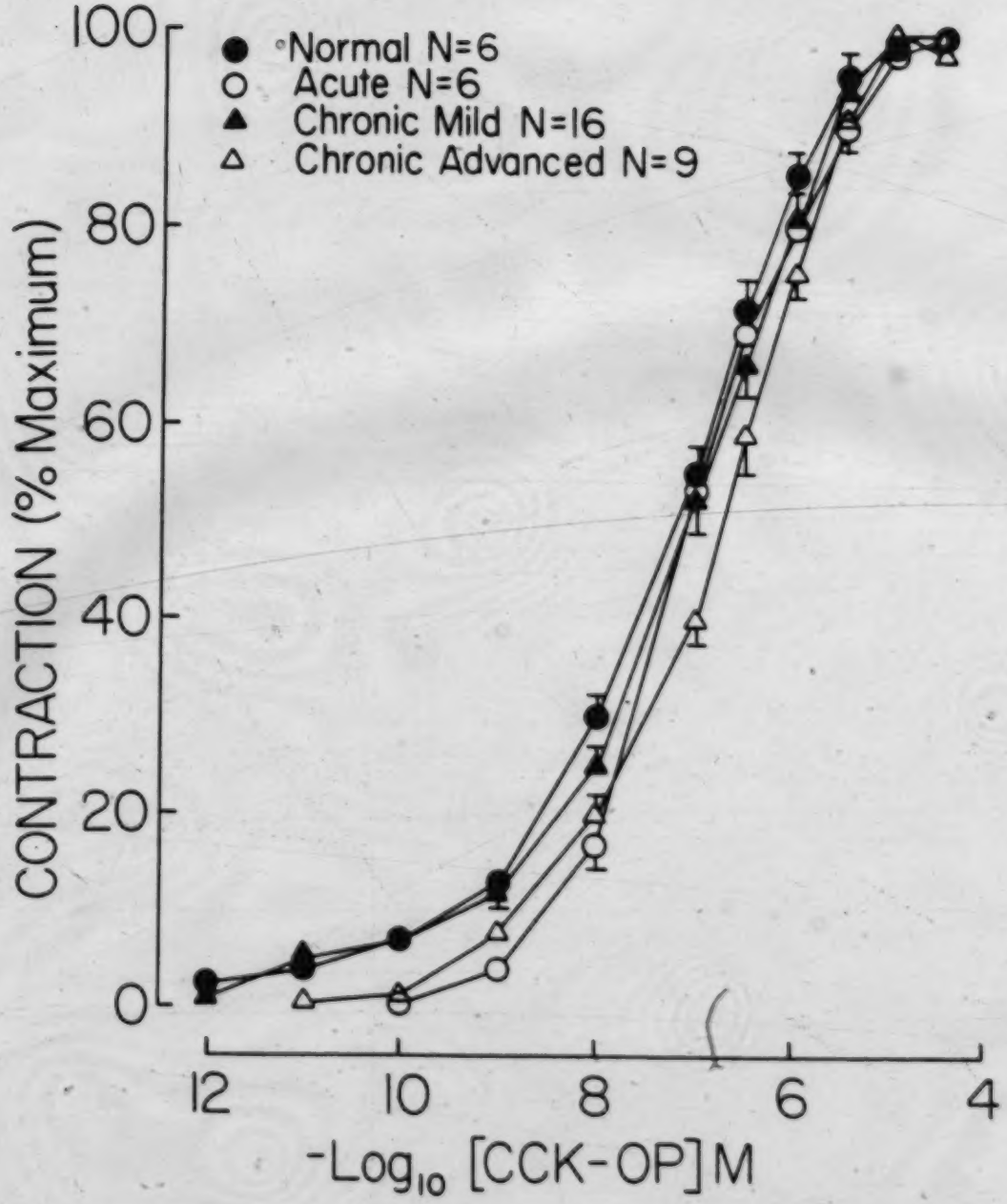
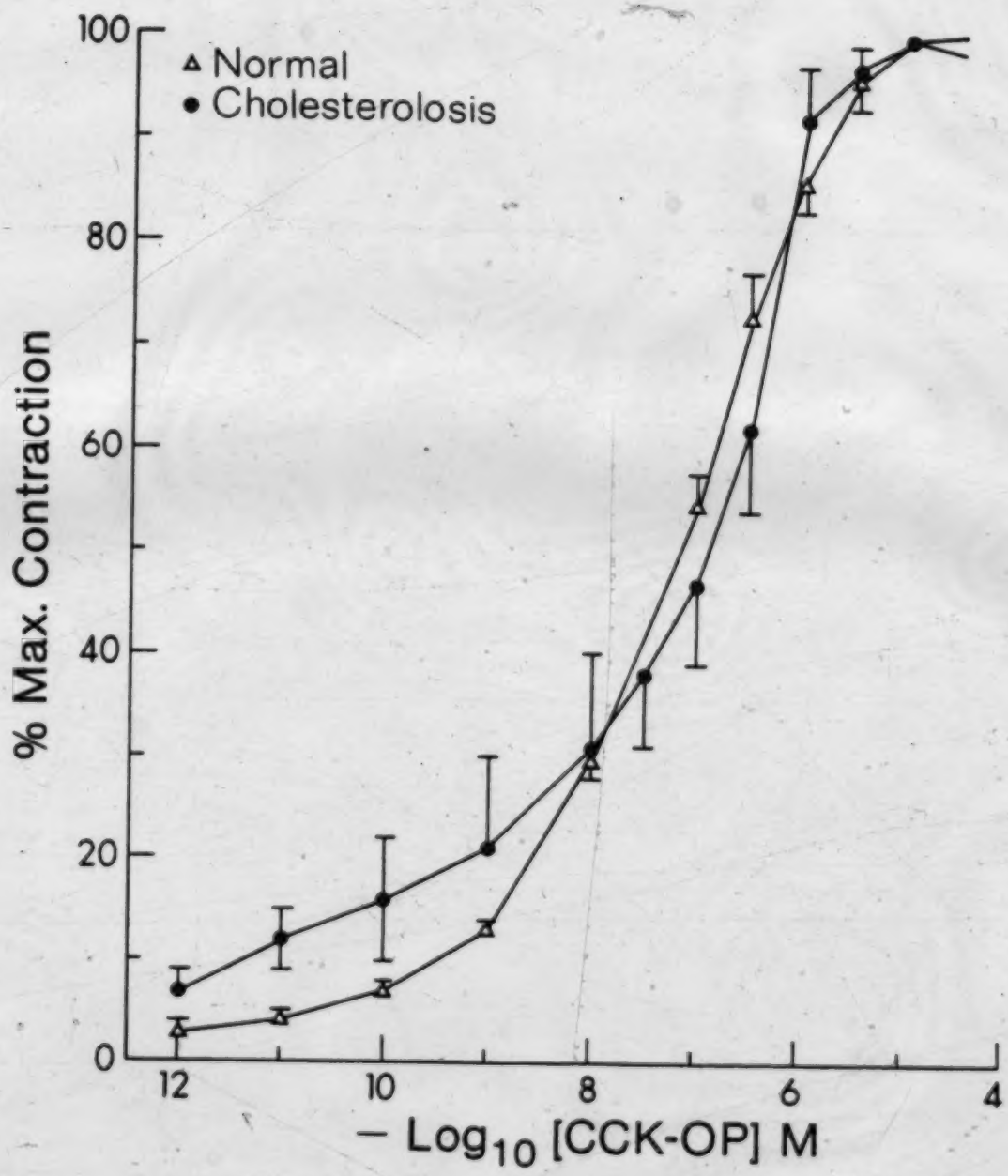

Normal N=6
Acute N=6
Chronic Mild N=16
Chronic Advanced N=9
CONTRACTION (% Maximum)
100
80
60
40
20
0
12
10
8
6
4
-Log10 [CCK-OP] M

FIGURE 23 : CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE

Contractile responses were expressed as a percentage of the maximal response of each strip. Curves are shown for strips from normal gallbladders (n=6) and those with cholesterolosis (n=3). Points represent the mean responses ± SEM.


100
80
60
40
20
0
△ Normal
● Cholesterolosis
% Max. Contraction
12
10
8
6
4
− Log10 [CCK-OP] M

chronic mild, chronic advanced, or acute cholecystitis (Table 3). Comparison of the maximal contractile responses in the different groups (Fig. 24,25), showed a significant decrease in the order of chronic mild cholecystitis, chronic advanced cholecystitis and acute cholecystitis. The maximum contractile responses in strips from normal gallbladders was less, but not significantly so, than the maximal responses obtained from strips with chronic mild cholecystitis, and was significantly greater than maximal responses in strips with chronic advanced and acute cholecystitis (Table 3). This relationship of responses, i.e. chronic mild cholecystitis > normal > chronic advanced > acute cholecystitis was the same for all CCK-OP concentrations (Fig. 24). Muscle thickness did not differ significantly among the disease groups studied but were significantly thicker than normal (Fig. 26).

Caerulein, Gastrin and Pentagastrin

Caerulein caused dose dependent contractions in strips from the eight gallbladders tested. Unfortunately, the highest concentration of the caerulein attainable in the bath was 0.1 nM and this did not cause a maximal contractile response. The characteristics of the caerulein-induced contractile response (i.e. onset of action, time to peak and relaxation) were similar to those of CCK-OP (Fig. 21). Like the CCK-OP contractile response the caerulein response was unaffected by atropine (100 μM) diphenhydramine (100 μM) or indomethacin (10 μM) but

TABLE 3 : Sensitivities and Contractile Responses to Cholecystokinin-octapeptide in normal and diseased groups.

GROUP	No.	ED_{50} (95% CONFIDENCE LIMITS) µM	CONTRACTILE RESPONSE (GRMS)
NORMAL	6	80 (35 - 182)	1.85 ± 0.26
CHOLESTEROLOSIS	3	99 (28 - 226)	2.10 ± 0.42
CHRONIC MILD	15	99 (58 - 166)	2.34 ± 0.34
CHRONIC ADVANCED	9	200 (75 - 538)	1.0 ± 0.08
ACUTE	7	160 (32 - 814)	0.33 ± 0.09

FIGURE 24 : CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE

Contractile responses are plotted for strips from normal gallbladders and those with chronic mild cholecystitis, chronic advanced cholecystitis, and acute cholecystitis. Points represent the mean response ± SD.

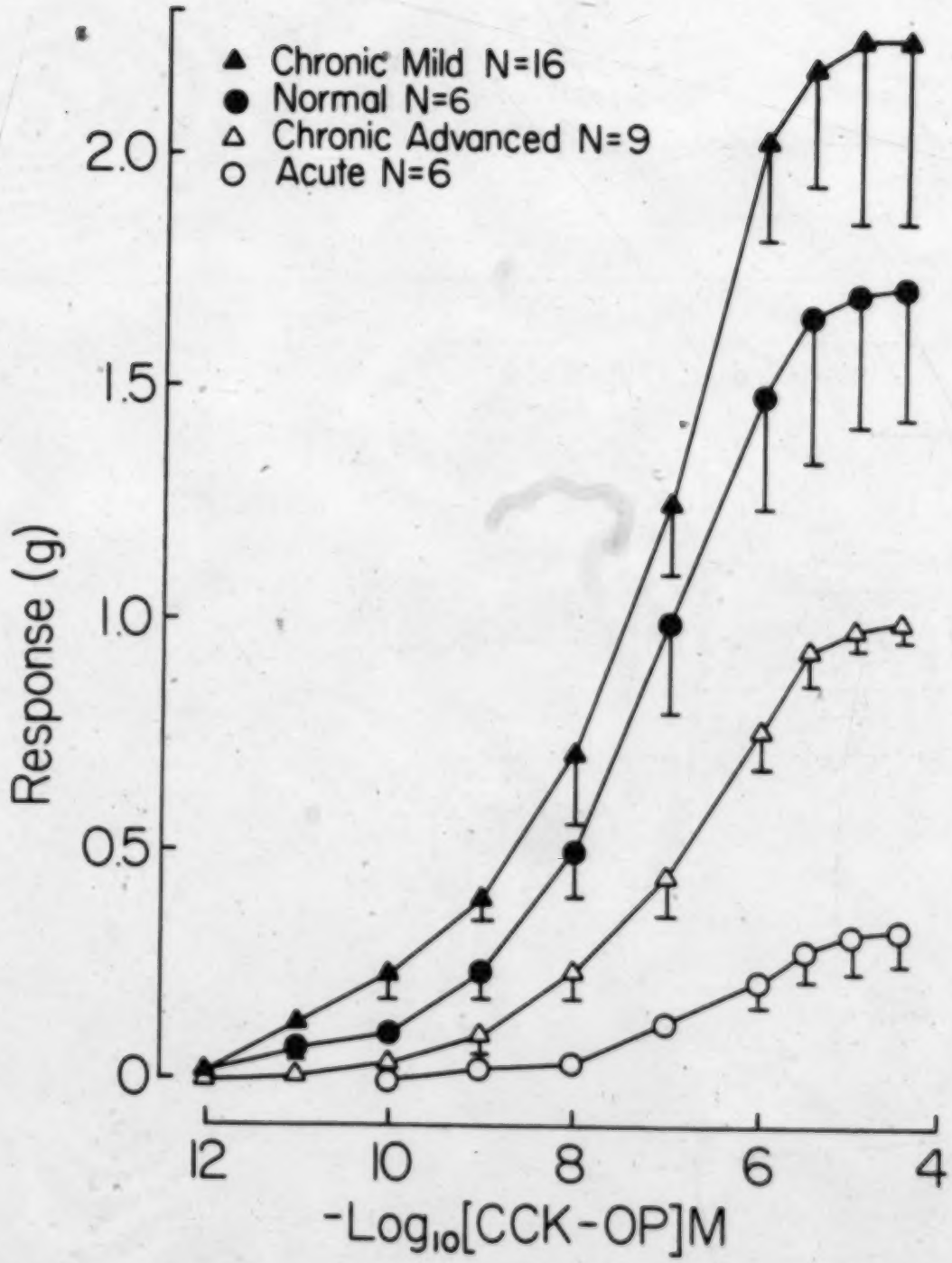

▲ Chronic Mild N=16
● Normal N=6
△ Chronic Advanced N=9
○ Acute N=6
Response (g)
2.0
1.5
1.0
0.5
0
12
10
8
6
4
-Log10[CCK-OP]M

FIGURE 25 : RELATIONSHIP BETWEEN HISTOLOGY SCORE AND CONTRACTILE RESPONSE TO CHOLECYSTOKININ-OCTAPEPTIDE

Points represent the mean maximal contractile response for strips from individual gallbladders plotted against the histology score for that gallbladder.

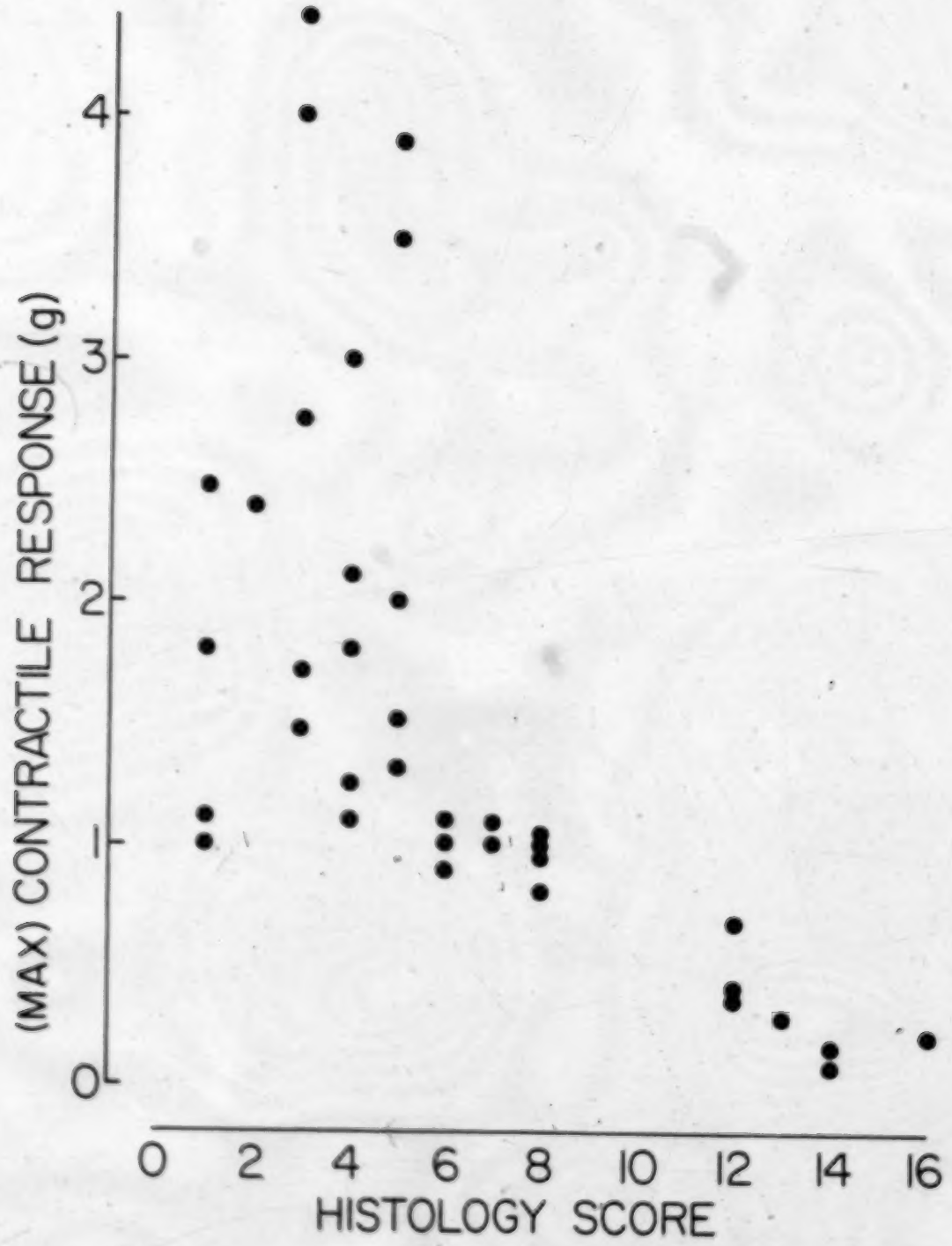
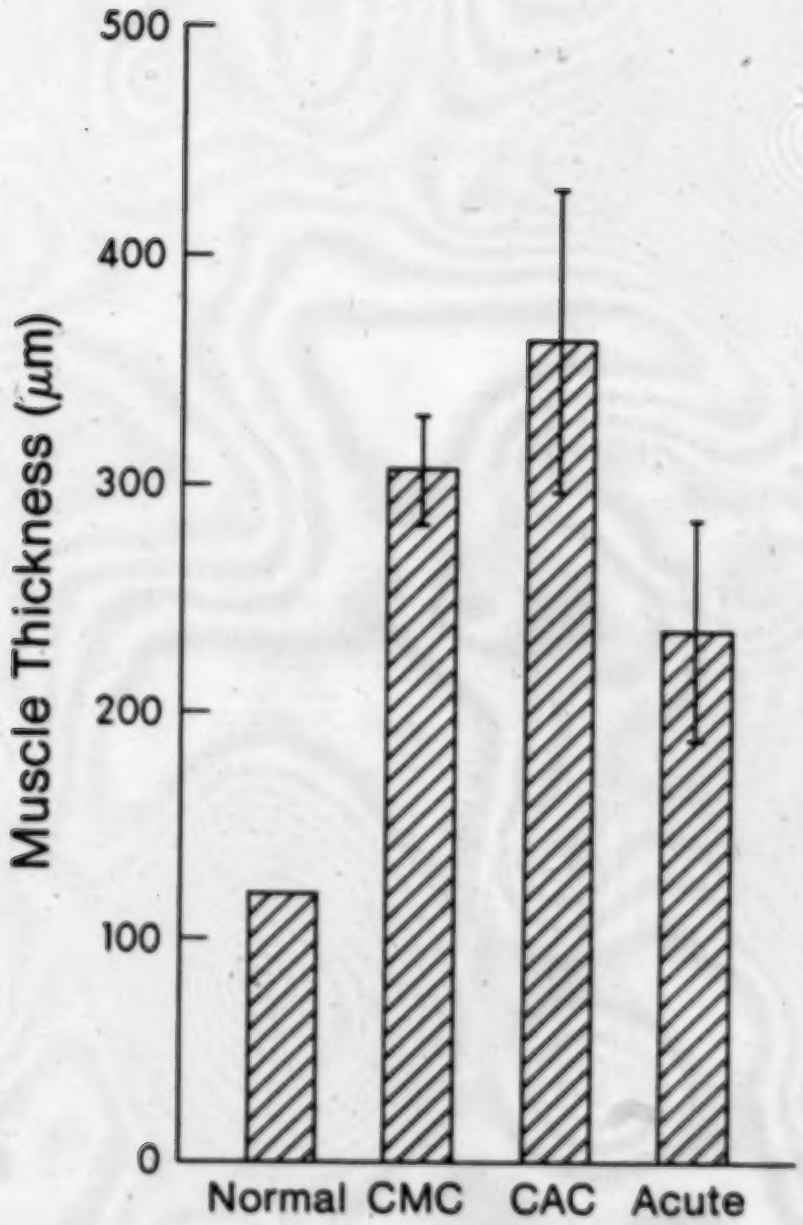

(MAX) CONTRACTILE RESPONSE (g)
0
1
2
3
4
0 2 4 6 8 10 12 14 16
HISTOLOGY SCORE

FIGURE 26 : MUSCLE THICKNESS IN GALLBLADDERS USED FOR CHOLECYSTOKININ STUDIES.

Histogram showing muscle thickness in normal gallbladders (n=6) and those with chronic mild cholecystitis (CMC, n=15), Chronic advanced cholecystitis (ACA, n=9) and acute cholecystitis (AC, n=6). Bars represent the standard deviation.


500
400
300
200
100
0
Muscle Thickness (μm)
Normal
CMC
CAC
Acute

was antagonised by dbGMP. The characteristics of the dbGMP antagonism of caerulein, i.e. effectiveness whether added before, with or after caerulein, spontaneous return of the contractile response on washing the strip with fresh Krebs, were similar to those of dbGMP antagonism of CCK-OP.

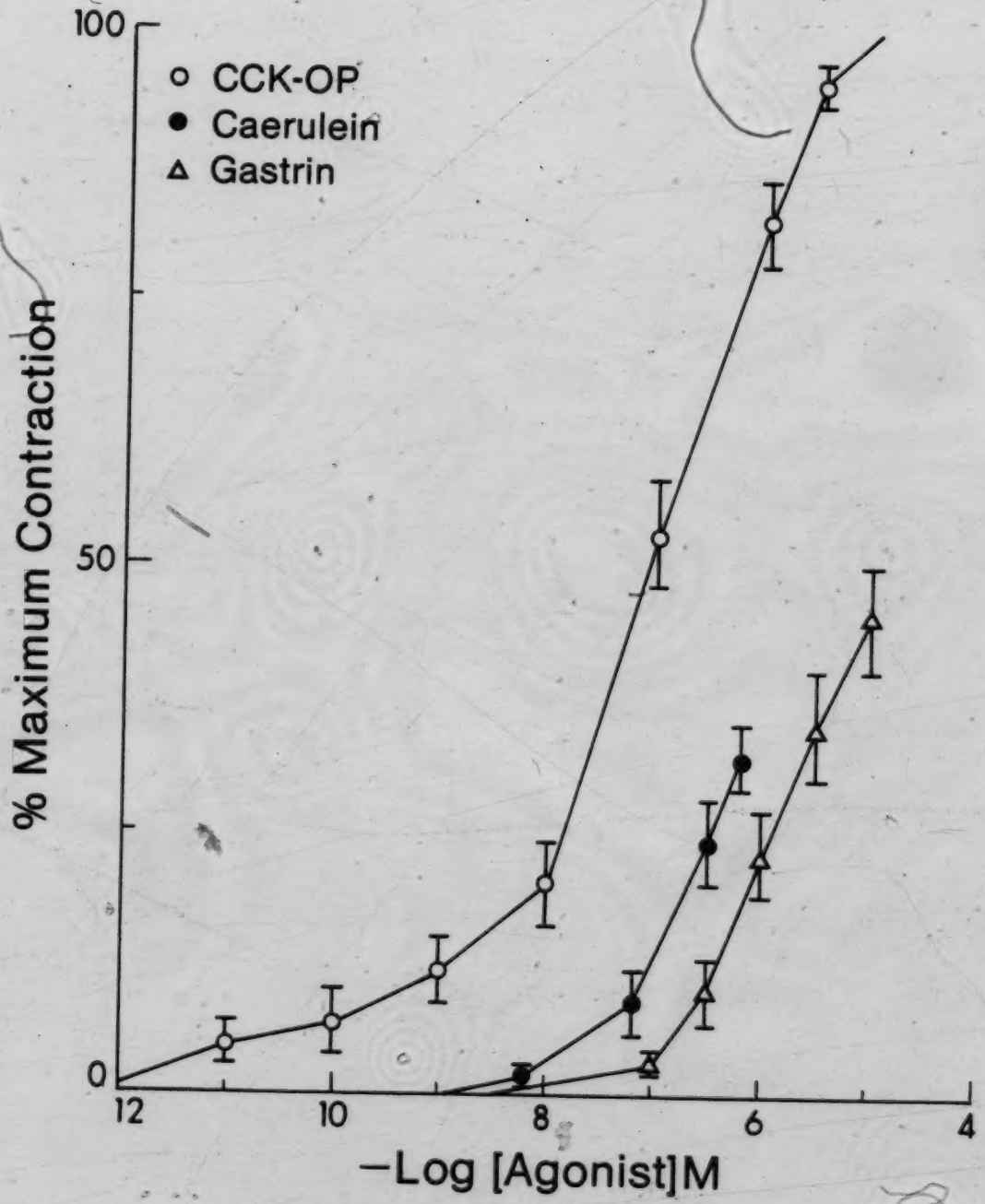
As a maximal contractile response was not elicited with the caerulein it was not possible to calculate the ED_{50}. A concentration-response curve was constructed by expressing the caerulein responses as percentages of the maximal CCK-OP responses in the strips. Based on this concentration-response curve (Fig. 27) caerulein was approximately 25 times less potent than CCK-OP.

The effects of Gastrin I and pentagastrin were studied in strips from 12 gallbladders and both caused concentration-dependent contractions in all gallbladder strips studied. Unfortunately it was not possible to obtain a Gastrin II preparation for this study. Gastrin and pentagastrin were of equal potency and efficacy on a molar basis with equal concentrations causing equal contractile responses in the same strip.

The characteristics of the responses were the same as those of CCK-OP and caerulein responses (Fig. 21) and like them were antagonised by dbGMP but not by other antagonists atropine, diphenhydramine or indomethacin. The contractile response to gastrin and pentagastrin 1 μM was abolished by the maximal attainable concentrations of dbGMP (300 μM): responses to higher concentrations were incompletely antagonised.

FIGURE 27 : CONCENTRATION-RESPONSE CURVES FOR CHOLECYSTOKININ-OCTAPEPTIDE, CAERULEIN AND GASTRIN/PENTAGASTRIN.

All responses were expressed as a percentage of the maximal CCK-OP response in that strip. Points represent the mean responses ± SEM.


100
50
0
% Maximum Contraction
CCK-OP
Caerulein
Gastrin
12
10
8
6
4
−Log [Agonist]M

When added to the bath together lower concentrations of CCK-OP and pentagastrin produced an additive contractile effect, and pentagastrin increased the tension in the CCK-OP contracted strips. With increasing CCK-OP concentrations the additive effect of gastrin decreased so that it had no effect on the maximal CCK-OP contraction (Fig. 28).

As with caerulein maximal contractile responses were not achieved with the gastrin or pentagastrin so that a true ED_{50} could not be calculated. Responses were therefore expressed as a percentage of the maximal CCK-OP responses. The greatest gastrin/pentagastrin effect achieved was 45% of the maximal CCK-OP produced response (Fig. 27). On a molar basis gastrin/pentagastrin was 100 times less potent than CCK-OP and 4 times less potent than caerulein (Fig. 27).

Substance P

The effect of substance P in concentrations of 1 nM to 30 μM was studied on 12 gallbladders and in these concentrations it produced concentration-dependent contractions. The onset of action was approximately 55 secs. which was much slower than with other contractile agents. The maximal contractile effect was reached after 60 secs and was usually caused by a concentration of 10 μM. The contractile response was small when compared to that of CCK-OP: the maximal response was 19 ± 4% of the maximal CCK-OP response.

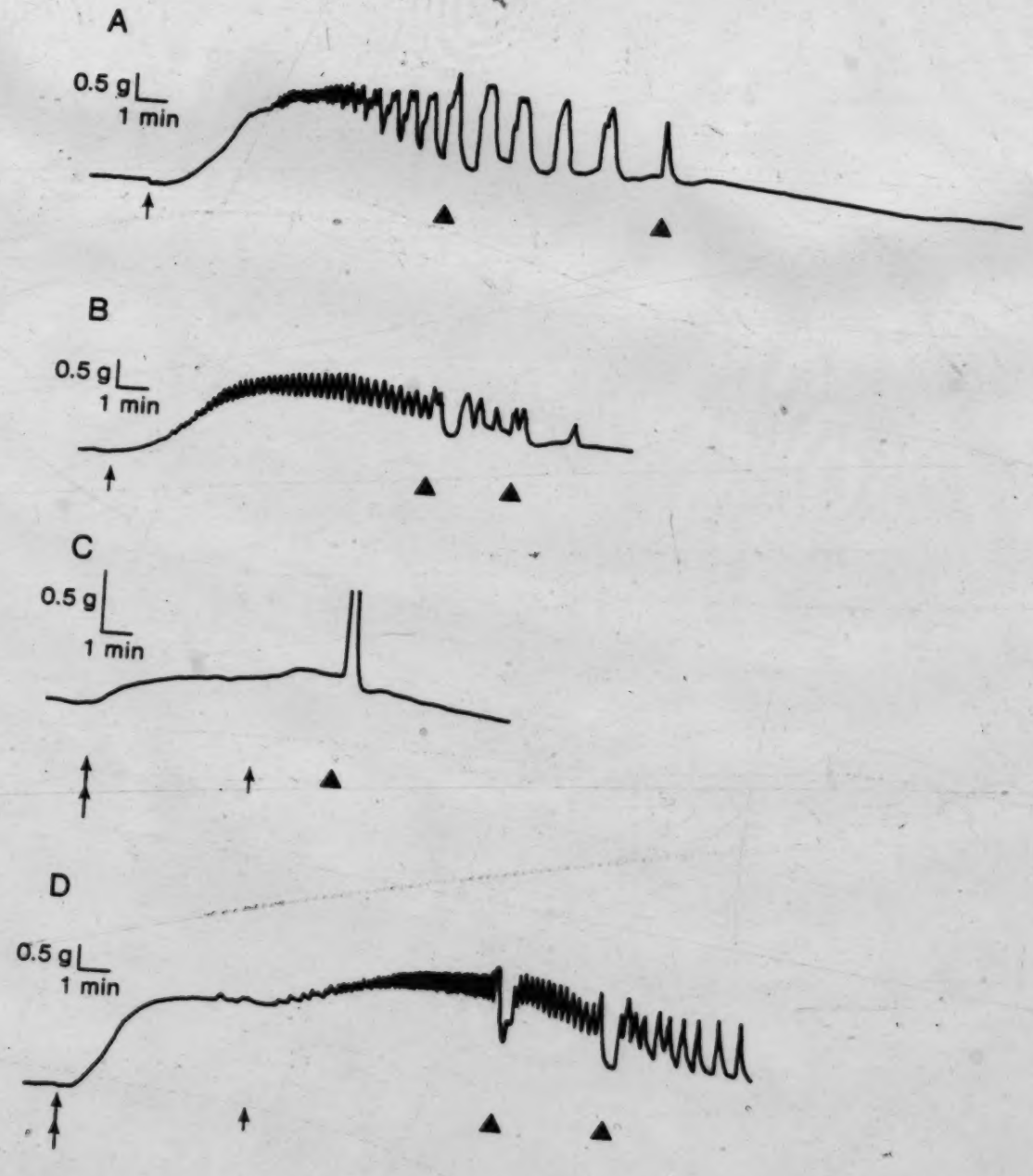
FIGURE 28 : RESPONSES TO PENTAGASTRIN

A & B — Tracings of contractile responses by gallbladder strips to pentagastrin (10 µM) (↑).

C — Tracings showing the additive effect of pentagastrin (100 nM) (↑) on a cholecystokinin-octapeptide (1 nM) (↑) contractile response.

D — Tracing showing the additive effect of pentagastrin (10 µM) (↑) on a cholecystokinin-octapeptide (100 nM) (↑) contractile response.

▲ Indicates washing with fresh Krebs' solution.


A
0.5 g
1 min
B
0.5 g
1 min
C
0.5 g
1 min
D
0.5 g
1 min

The effects os substance P when combined with low concentrations of CCK-OP were additive (Fig. 29). However, this additive effect decreased as concentrations of CCK-OP were increased and was not evident with maximal CCK-OP concentrations.

The contractile effect of substance P was unaffected by the muscarine receptor antagonist, atropine (100 μM), the histamine H_1 receptor antagonist diphenhydramine (100 μM) or the CCK-OP antagonist dbGMP (300 μM). The Substance P concentration-response curve is shown in Fig. 30.

Vasoactive intestinal polypeptide (VIP)

As the results of stimulating human gallbladder with VIP were unexpected the method and drug potency were assessed on guinea pig gallbladder strips as this is a proven experimental model for this agent (103).

Human gallbladder

Spontaneous activity developed in 32 of 40 human gallbladder strips studied. Concentration-related contractile responses were elicited by cholecystokinin-octapeptide CCK-OP (1 pM to 1 μM). VIP (1 pM to 1 μM) had no noticeable effect on spontaneous activity nor did it affect the resting tension of the strips. Submaximal concentrations of CCK-OP caused concentration-dependent increments in tension which were unaltered by the addition of VIP (1 pM to 1 μM) to the bath prior to CCK-OP or during the plateau phase of the CCK-OP response.

FIGURE 29 : EFFECTS OF SUBSTANCE P

A,B & C — Tracings showing the contractile effects of substance P (10 μM) (↑) on gallbladder strips.

D — Tracing showing the additive effect of substance P (1 μM) (↑) on the CCK-OP (10 nM) (↑) contractile effect.

▲ Indicates washing with fresh Krebs' solution.

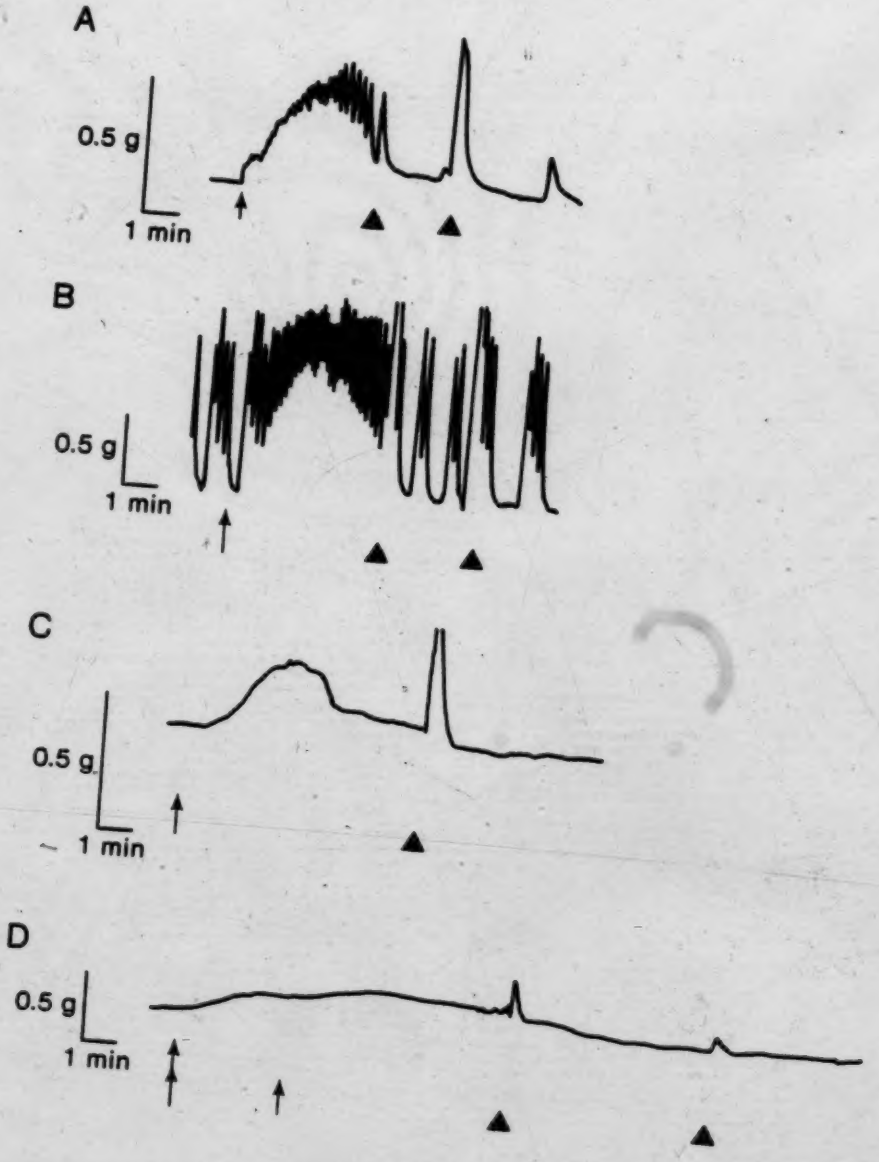
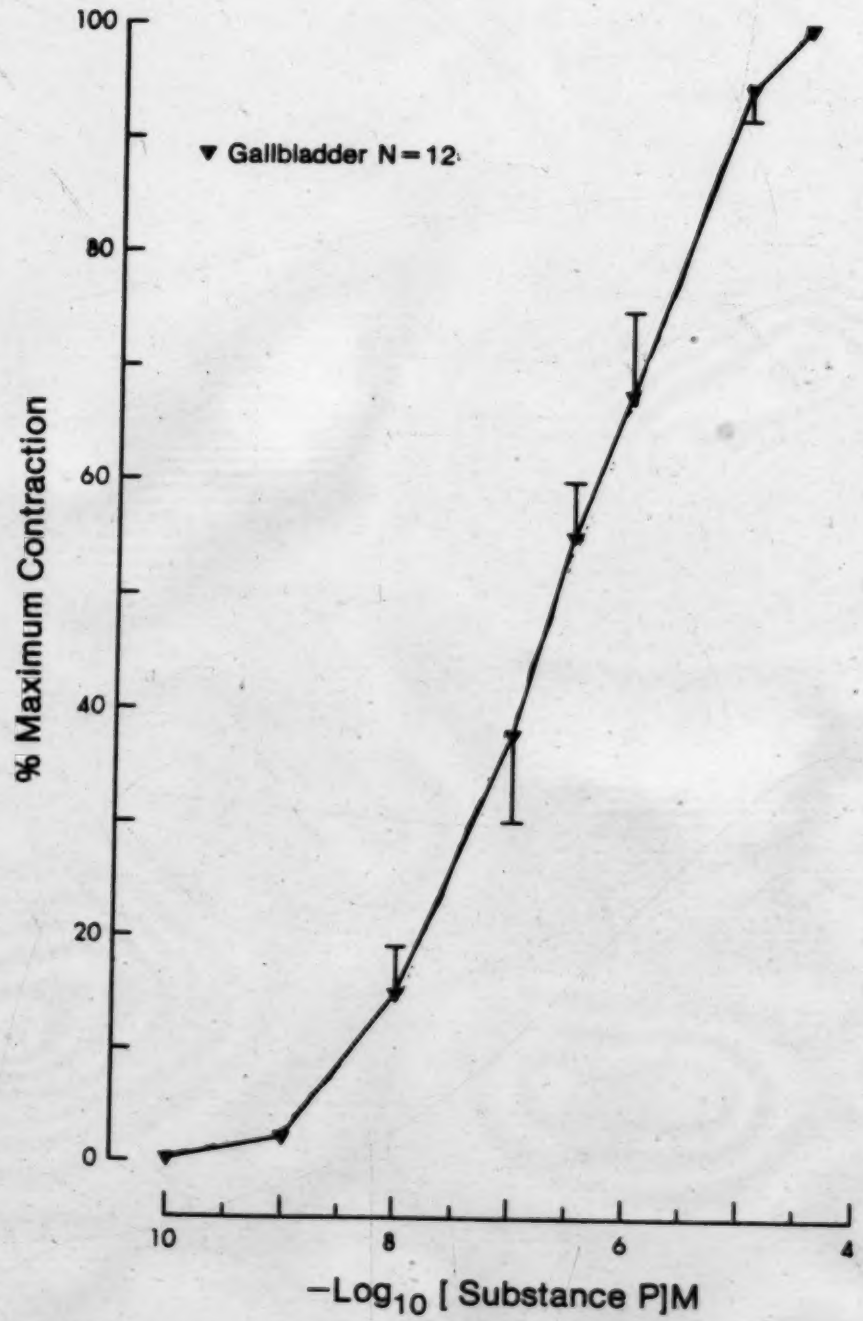

A
0.5 g
1 min
B
0.5 g
1 min
C
0.5 g
1 min
D
0.5 g
1 min

FIGURE 30 : CONCENTRATION-RESPONSE CURVE FOR SUBSTANCE P.

Responses in each gallbladder strip were expressed as percentages of the maximum contractile response in that gallbladder strip. Points represent the mean ± SEM.


▼ Gallbladder N = 12
% Maximum Contraction
100
80
60
40
20
0
10
8
6
4
−Log10 [Substance P]M

Guinea pig gallbladder

In 33 (74%) guinea pig gallbladders, VIP, in concentrations of 0.1 pM to 0.1 nM, caused concentration-related contractions (Fig. 31, 32) and concentrations of 1 nM to 10 nM caused smaller contractions. Still higher concentrations (100 nM to 1 uM) caused relaxation of all gallbladder strips (Fig. 31). The contractile responses were rapid in onset and rapidly reached a peak (30s) whereas the relaxant resonses, though equally rapid in onset, reached maximum relaxation more slowly (2 min). The maximum contractile response to VIP was small and was approximately 16% of the maximum response to CCK-OP (Fig. 33). Spontaneous activity, which developed in all strips, was unaffected by VIP except where there was marked relaxation of the strips. The contractile effect of VIP was unaffected by the muscarine receptor antagonist, atropine (10 μM), the histamine H_1 receptor antagonist, diphenhydramine (10 μM) or the prostaglandin synthetase inhibitor indomethacin (10 μM).

The contractile responses to low concentrations of CCK-OP (0.01 pM - 1 pM) in combination with low concentrations of VIP (0.1 pM - 10 pM) were apparently additive (Fig. 34). As the tone was increased with higher concentrations of CCK-OP, the contractile effect of VIP decreased regardless of VIP concentrations. Concentrations of VIP which decreased resting tension also relaxed CCK-OP contracted strips. The

FIGURE 31 : EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON GUINEA PIG GALLBLADDER.

Portions of a typical recording showing the effects of increasing concentrations of VIP on the tone and spontaneous activity of guinea pig gallbladder strips. In the top panel VIP 1 pM (left +), 10 pM (centre +) and 100 pM, (right +), cause increasing contractile response while in the bottom panel VIP 100 nM (left +) and 1 uM (right +) cause relaxation.

▲ Indicates washing with fresh krebs solution.

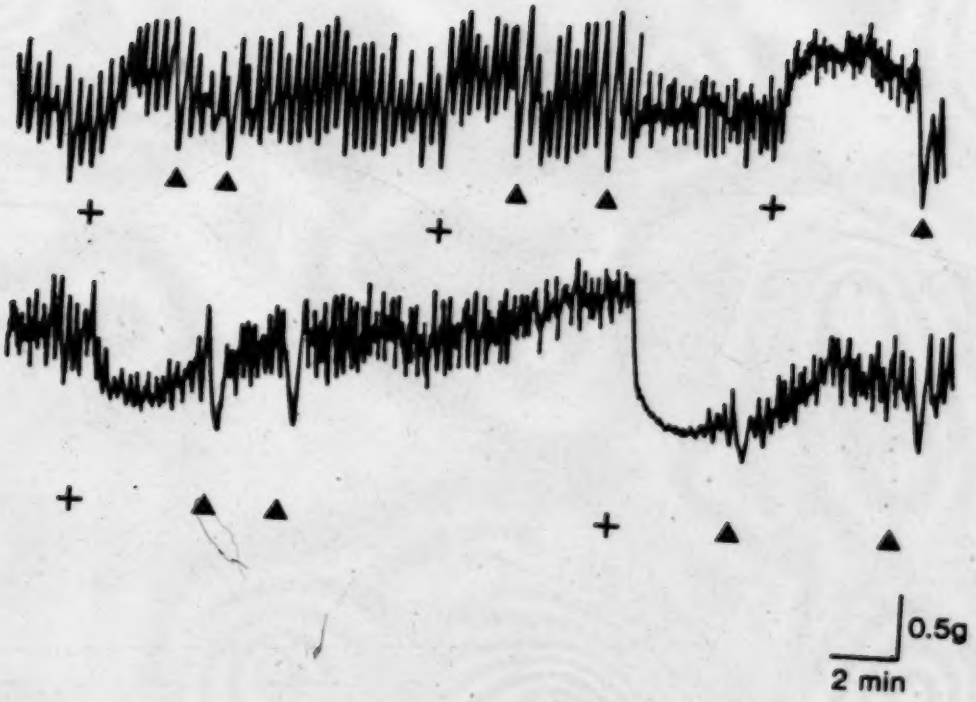

0.5g
2 min

FIGURE 32 : CONCENTRATION - RESPONSE CURVE FOR VASOACTIVE INTESTINAL POLYPEPTIDE IN GUINEA PIG GALLBLADDER.

Contractile responses are expressed as a percentage of the maximal response to VIP (approximately 16% of the maximal response to CCK-OP). Relaxant responses are calculated as a percentage change in resting tone (initially set to 1 g). Points represent the mean ± SEM, n = 33.

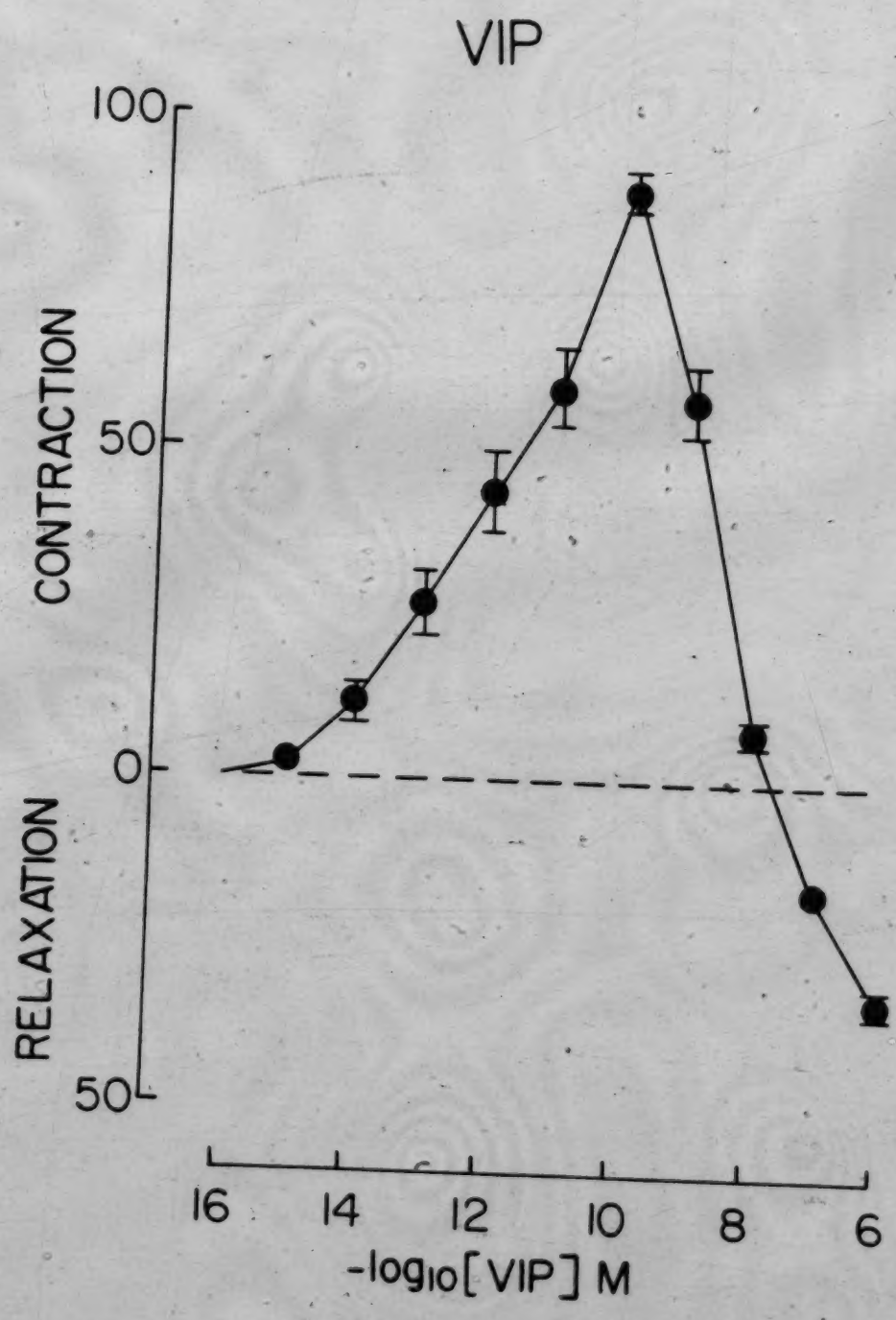

VIP
100
50
0
50
CONTRACTION
RELAXATION
16
14
12
10
8
6
-log10[VIP] M

FIGURE 33: CONCENTRATION-RESPONSE CURVES FOR VASOACTIVE INTESTINAL POLYPEPTIDE AND CHOLECYSTOKININ-OCTAPEPTIDE IN GUINEA PIG GALLBLADDER

Mean contractile responses by gallbladder strips to CCK-OP (n=8) and VIP (n=40) were plotted. Points represent the mean contractile responses ± SEM.

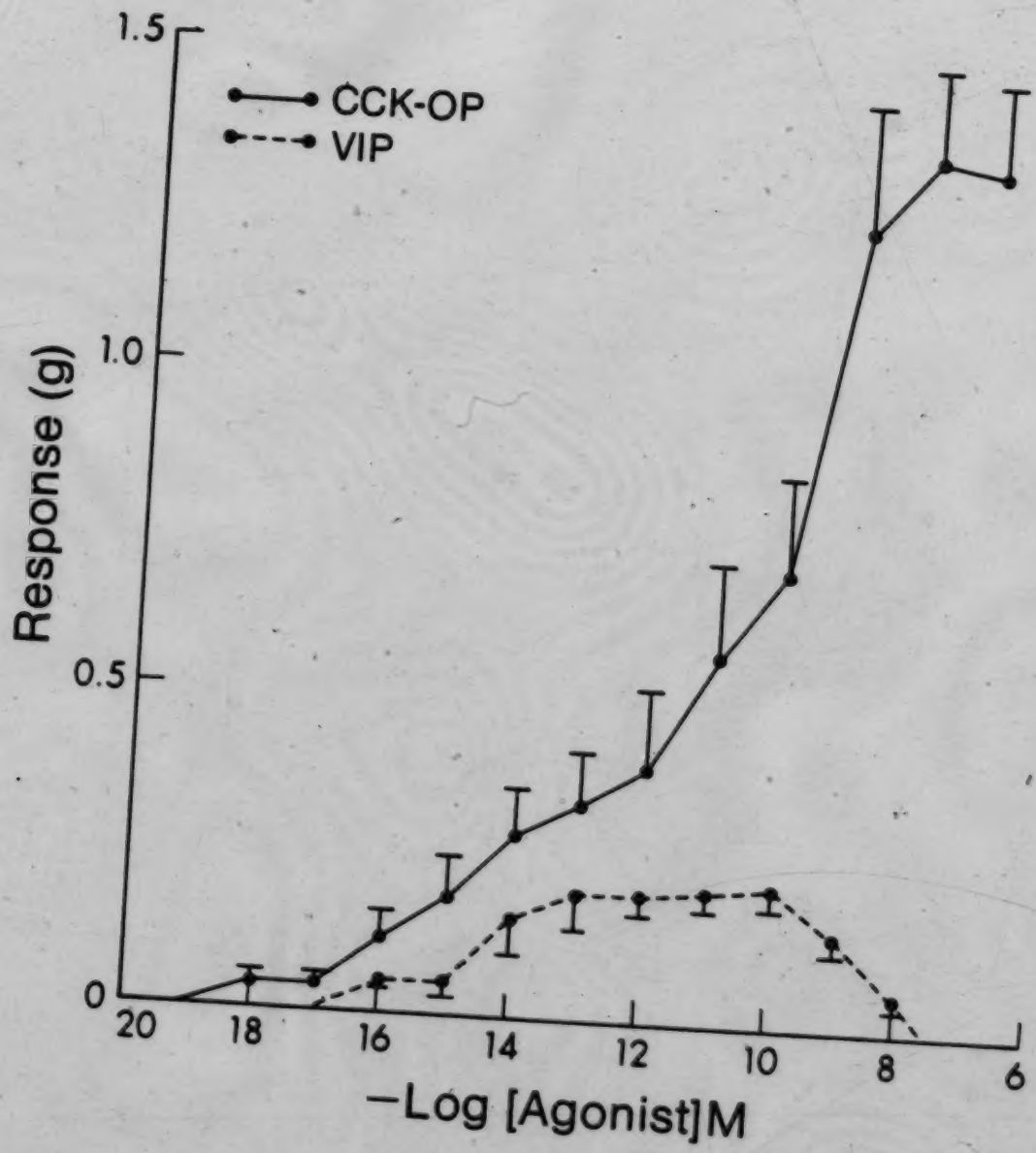
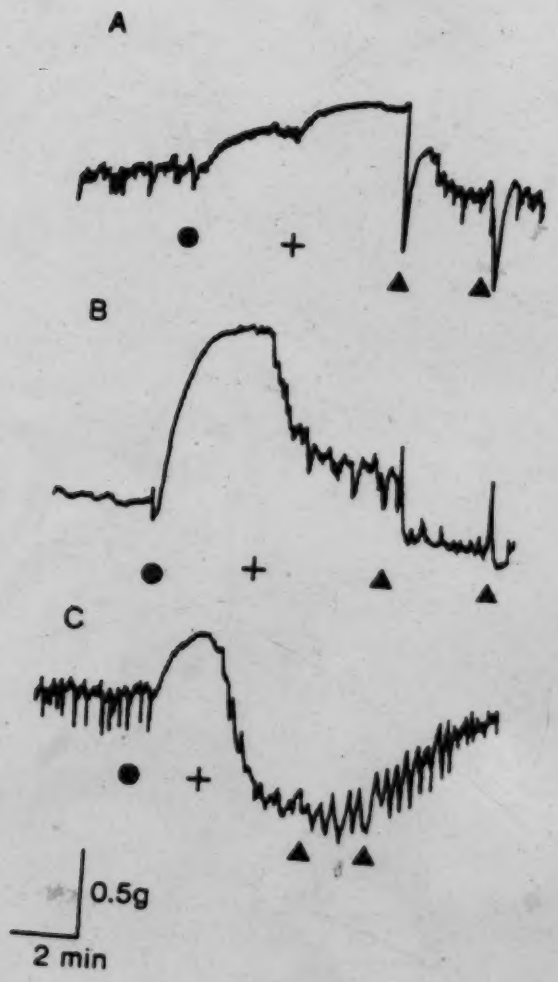

1.5
1.0
0.5
0
Response (g)
CCK-OP
VIP
20
18
16
14
12
10
8
6
−Log [Agonist]M

FIGURE 34 : EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON CHOLECYSTOKININ-OCTAPEPTIDE CONTRACTED GUINEA PIG GALLBLADDER.

Portions of a typical recording showing the effect of various concentrations of VIP on responses of guinea pig gallbladder strips elicited by CCK-OP. In Panel A, following a small contraction to CCK-OP (10^{-12}M), 10^{-10}M VIP (+) induced an increase in tone, in B 10^{-7}M VIP (+) reduced the stimulatory effect of a higher concentration (10^{-10}M) of CCK-OP whereas, in C, the contractile effect of 10^{-11}M CCK-OP was abolished by 10^{-6}M VIP (+) and relaxant response was obtained.

▲ Indicates washing with fresh Krebs' solution.


A
B
C
0.5g
2 min

contractile responses to low concentrations of CCK-OP (0.01 pM - 10 pM) were abolished by 1 μM and in some instances, were converted to relaxations. With increasing CCK-OP concentrations the relaxant effect of high concentrations of VIP decreased so that the maximal contractile effect of CCK-OP (10 nM) was unaffected by VIP (1 μM).

Secretin and other hormones

One preparation of secretin (Sigma) caused contractile responses which were believed due to CCK contamination of the preparation. The characteristics of the responses were similar to those of CCK-OP. A pure secretin preparation had no demonstrable effect on spontaneous activity, resting tone, or CCK-OP induced tone.

None of the following hormones had any demonstrable effect on the resting tension, spontaneous activity or CCK-OP induced tension in gallbladder strips: glucagon (1 - 100 μM), motilin (1 nM _ 3 μM), vasopresin (0.1 - 10 units/ml), neurotensin (10 nM - 30 μM), and bombesin (1 mM - 30 μM).

SUMMARY OF RESULTS

1. Cholesterolosis and cholesterol gallstones frequently co-existed.

2. Histological findings were similar in calculous and acalculous chronic mild cholecystitis.

3. All diseased gallbladders, calculous and acalculous cholecystitis, had significant muscle hypertrophy. The degree of hypertrophy was simular in both groups.

4. Gallbladder sensitivity to cholecystokinin-octapeptide was unaltered in cholesterol gallstone disease or acalculous cholecystitis.

5. Glucagon, secretin, motilin, neurotensin and bombesin had no direct action on gallbladder motility.

6. The gallbladder contractile response to histamine was due to H_1 receptor stimulation. There was a very small histamine H_2 receptor population in the human gallbladder.

7. Gallbladder sensitivity to histamine stimulation increased with increasing degrees of inflammation.

8. Vasoactive intestinal polypeptide had no effect on human gallbladder <u>in vitro</u> while in the guinea pig low concentrations caused contractile responses and

higher concentrations caused relaxation of resting and CCK-OP induced tone.

9. Human gallbladder contained only a small population of α-adrenergic receptors, stimulation of which caused contraction.

10. The predominant effect of adrenergic stimulation was muscle relaxation which was mediated by β_2 adrenergic receptors.

11. Nonadrenergic - noncholinergic inhibition was not demonstrated in human gallbladder strips.

DISCUSSION

Normal Gallbladders

As outlined in Results, gallbladders were classified as normal only if strict criteria were fulfilled. Of the 6 normal gallbladders 4 were excised because of false positive radiological reports. When one examines the clinical history of these patients it is clear, as outlined in Table I, that their symptoms were minimal. Histologically, scattered inflammatory cells, mainly lymphocytes were seen in the walls of these gallbladders. Carman et al (148), who examined 5,000 consecutive gallbladders obtained at necropsy, and Mackay (143) have stated that the finding of scattered inflammatory cells in the wall of the gallbladder does not constitute an abnormality.

The most significant objective difference between these 6 gallbladders and the diseased gallbladders was in muscle thickness. Muscle thickness in the 6 normal organs fell within an 8 μm range (116-123), all being at least 50% thinner than the thinnest diseased gallbladder. There was, however, one diseased gallbladder in which muscle thickness was normal. This gallbladder contained pigment calculi which were secondary to hereditary spherocytosis which necessitated splenectomy.

It is justified to classify these 6 gallbladders as normal for the following reasons;

1. Minimal symptoms - not clearly biliary.
2. No calculi present.
3. Normal extrahepatic biliary tree.
4. No inflammatory changes in the gallbladder wall.
5. No muscle hypertrophy.
6. No cholesterolosis.
7. No Rokitansky-Aschoff sinuses.

Cholecystitis

There was little evidence of inflammatory changes in the gallbladders with chronic mild cholecystitis. The histological abnormality in this group consisted mainly of mucosal hyperplasia, with the formation of mucosal polyps, and muscle hypertrophy. These findings were present in all gallbladders in this group.

In most specimens with chronic mild cholecystitis there were scattered inflammatory cells at the muscle and adventitional interface. This is not considered to be an abnormality (143,148). Inflammatory changes in the mucosal layer, if present, were minimal. These findings are in keeping with the current concept that gallstones could theoretically form in a histologically (i.e. no inflammation) normal gallbladder. Microscopic findings in this stage are in agreement with those of Levine (20) in the stage referred to by her as the hypertrophic stage.

With increasing severity of disease there was increasing

evidence of inflammation in all the layers of the gallbladder wall.

All specimens with acute cholecystitis were in fact due to an acute inflammatory episode as evidenced by polymorphonuclear cell infiltration, red cell extravasation and edema, superimposed on chronic inflammation as evidenced by mucosal atrophy and fibrosis. There was no gallbladder in this study with true acute cholecystitis. In a study of several hundred surgically removed gallbladders, Levine (20) found no organ with acute cholecystitis.

Acalculous Cholecystitis

In this study there were 5 gallbladders with acalculous cholecystitis. As in the chronic mild cholecystitis group there were only scattered inflammatory cells, mainly lymphocytes, at the muscle-adventitial interface, which does not constitute an abnormality. Use of the term 'cholecystosis' rather than cholecystitis as suggested by Jutras et al (144) and Goldstein et al (145) is therefore more correct.

All 5 gallbladders had cholesterolosis, in 4 it was of a granular type, sometimes referred to as the strawberry gallbladder because of its resemblance to a very ripe strawberry, and in the other specimen the cholesterolosis was more polypoid in appearance. Cholesterolosis also occurred in associated with cholelithiasis in 6 cases, i.e. an incidence of 14%. This is in agreement with

Illingworth (149) who stated that pure cholesterol gallstones are frequently present in association with cholesterolosis and is in contrast to Berk et al (147) who stated that there was no relationship between cholesterolosis of the gallbladder and supersaturation of cholesterol in bile or cholesterol gallstones. Several other studies have reported cholesterolosis in 10 - 15% of patients with gallstones (152,178). It is interesting that cholesterolosis was not present in any gallbladder with advanced cholecystitis. This concurs with the statement by Lahey (141) in 1927, that "...the cholesterol deposits of the so-called 'strawberry' type tend less and less to appear as the inflammatory process in the gallbladder wall increases". This may be due to removal of the cholesterol deposit by the inflammatory process.

Another finding in acalculous cholecystitis were the Rokitansky-Aschoff sinuses which were present in 4 of the 5 acalculous cholecystitis gallbladders. Rokitansky-Aschoff sinuses together with proliferation of muscle and epithelium compose the 3 features of adenomyomatosis as defined by Jutras (144) and referred to as cholecystitis glandularis proliferans (179).

Berk et al (147) stated that cholesterolosis and adenomyomatosis are 2 separate gallbladder diseases with differing etiologies. In this study all 5 organs with cholesterolosis and 6 with cholesterolosis and cholesterol gallstones had mucosal hyperplasia and muscle hypertrophy and Rokitansky-Aschoff sinuses were present in 8 of these

gallbladders. Although the number with acalculosis cholecystitis is small these findings suggest that cholesterolosis and adenomyomatosis are not different diseases and may have the same aetiology.

Acalculous and Calculous Cholecystitis: The Same Disease?

Muscle hypertrophy was present in all gallbladders with cholesterol gallstones and all with cholesterolosis. Cholesterolosis was present in 14% of gallbladders with cholesterol gallstones and cholesterol gallstones were present in 55% of gallbladders with cholesterolosis. Rokitansky-Aschoff sinuses were present in four of six gallbladders with cholesterolosis, and 64% of gallbladders with cholesterol gallstones. This is similar to the findings of Elfving (152) who reported sinuses present in 86% of chronic cholecystitis gallbladders, in 95% of which there were associated gallstones.

These findings suggest a strong association between cholesterolosis, Rokitansky-Aschoff sinuses, muscle hypertrophy, and cholesterol gallstones. This is in contrast to the statement by Berk et al (147) that there was no association between cholesterolosis and Rokitansky-Aschoff sinuses and that the two conditions were not related. As previously suggested (180) the muscle hypertrophy and Rokitansky-Aschoff sinuses may result from resistance to gallbladder outflow and there is good evidence (17) that, at least in

the prairie-dog model, there is increased cystic duct resistance in association with cholesterol gallstone disease.

It is, therefore, proposed that cholesterol gallstones and acalculous cholecystitis represent different presentations of the same condition, the etiology of which is multi-factorial and increased cystic duct resistance is one of these factors.

GALLBLADDER MOTILITY

Spontaneous activity has previously been described in gallbladder body strips which were diseased (15). In this study spontaneous activity was noted in normal gallbladder tissue as well as in diseased. The inhibitory effect of the prostaglandin synthetase inhibitor indomethacin on spontaneous activity, as reported by Lennon (15), has been confirmed in this study. Inhibition of guinea pig gallbladder strip spontaneous activity has been previously reported by Doggrell and Scott (181) and in this study spontaneous activity was abolished by indomethacin. Inhibition of spontaneous activity and suppression of basal tone, by indomethacin has previously been reported in human longitudinal ileal muscle (182). These findings suggest that prostaglandins play a regulatory role in spontaneous activity.

The slight but definite inhibitory effect by the histamine H_1 receptor antagonist diphenhydramine on spontaneous activity suggests that histamine may also be

involved in this regulation.

Analysis of Responses

Contractile and relaxant responses are usually expressed as force per unit cross-sectional area or unit mass. This was not done in this study for the following reasons: Strips are composed of muscle, connective tissue, and mucosa and muscle bundles are not aligned longitudinally but are arranged in a meshwork. As the mucosa is adherent to the muscle layer, and the connective tissue is interspersed between the muscle bundles, it is not possible to get strips composed solely of muscle tissue. As the muscle bundles are arranged in a multidirectional fashion each contractile response is the sum of vectors of individual muscle bundles acting along different axes. The problem is compounded when working with diseased gallbladders as muscle content in the gallbladder wall may vary from 2.7 to 35.3% (15) and also there is muscle fibrosis. For these reasons contractile responses were not expressed as tension developed per strip: strips were of equal size. For sensitivity studies individual responses in each strip to a given agent were normalized by expressing them as percentages of the maximal response by that strip to that agent.

NON-HORMONAL REGULATION

Acetylcholine

The results of this study confirm that human gallbladder possesses cholinergic receptors. The finding that the gallbladder sensitivity to acetylcholine did not differ between chronic mild and chronic advanced disease suggests that sensitivity is not altered in diseased tissue.

There was no difference between the magnitude of contractile responses in the two disease groups which is surprising, for although the muscle hypertrophy was similar in both groups there was significant fibrosis in the chronic advanced group. Also the contractile response to cholecystokinin octapeptide in the chronic mild group was significantly greater than that in the chronic advanced group. Due to insufficient tissue, concentration-response curves were not constructed for acetylcholine in normal tissue. In guinea pigs on a cholesterol diet gallbladder contractility to acetylcholine has been shown to be significantly greater than in control animals (163). However, in that study the muscle content of strips was not measured and the increased response may have been due to muscle hypertrophy. It is possible that responses to acetylcholine would be greater in a diseased group as was true with cholecystokinin.

Adrenergic Receptor Stimulation

While a significant α-receptor population has been identified in feline (54) and guinea pig (56) gallbladder a very small α-receptor population was demonstrated in less than one quarter of the gallbladders in this study. In the gallbladder the α-receptors mediate contractions in the gallbladder in contrast to most intestinal smooth muscle in which α-receptor-mediated relaxation occurs (183). As a very small α-receptor population was demonstrated in only a few gallbladders it is unlikely that they play any significant role in biliary motility. Conversely β-adrenoreceptors were identified in all gallbladders studied whereas others have identified them in less than half feline (54) and human (79) gallbladders. The β-adrenergic-receptor mediated relaxant effect was most evident in cholecystokinin-precontracted strips and may play a role in the regulation of gallbladder motility.

That the β-relaxant effect was inhibited by the β-receptor antagonist propranolol, and not by the selective β_1 antagonist practolol, suggests that the receptors are of the β_2 variety.

Nerve Stimulation

Field stimulation is used in-vitro studies to stimulate nerve stimulation. That resultant contractile responses were due to nerve stimulation was confirmed by its inhibition by atropine.

The contractile responses to field stimulation in this study were small when compared to the contractile response to acetylcholine and cholecystokinin stimulation. The vagus provides the biliary tract with motor fibres and vagal stimulation causes an increase in intragallbladder pressure, which is insufficient to expel bile from the gallbladder (184). Perhaps this is reflected in this study by the relatively poor contractile response to field stimulation. Failure of the gallbladder to empty in response to vagal stimulation may also be due to concurrent vagal stimulation and resultant contraction of the cystic duct musculature (180).

Field stimulation in the presence of atropine simulates adrenergic nerve stimulation and in this study caused relaxation of the gallbladder strips. This is in agreement with reports by Persson (55) who demonstrated gallbladder relaxation in response to splanchnic nerve stimulation in-vivo, particularly during contraction in response to cholecystokinin. The relaxant effect was inhibited by the β-receptor antagonist propanolol which in a very small number of strips unmasked a small contractile response. This small contractile response was probably due to α-receptor stimulation as the addition of acetylcholine to the bath had no effect.

Field stimulation in the presence of both atropine and propanolol did not produce relaxation in any strips,

i.e. noncholinergic-nonadrenergic inhibition was not demonstrated. Noncholinergic-nonadrenergic inhibition has been reported in the guinea pig gallbladder (63,64) but results of this study suggest that it is not a factor in human gallbladder motility.

Histamine

There have been several reports of histamine induced contractile responses in guinea pig (185), baboon (126) and human (15) gallbladder. The findings in this study are in total agreement with those of Lennon (15) who reported an alteration in gallbladder sensitivity to histamine among varying degrees of cholecystitis. An important additional finding in this study is the extension of this observation to normal tissues: i.e. there is a gradual increase in sensitivity from normal to acutely inflamed tissues. There was a 30-fold increase in sensitivity from normal gallbladders to those with acute cholecystitis. With the use of the histological scoring system, we found a significant correlation ($P < 0.01$) between the degree of cholecystitis and the sensitivity of the muscle strips to histamine. The mechanism underlying this changing sensitivity to histamine is as yet unclear but could be due to altering histamine receptor affinity, or an interaction with inflammatory agents such as prostaglandins as suggested by Lennon (15). It is also possible that in cholecystitis

there may be increased endogenous histamine production by mast cells: this would cause a false increase in sensitivity and could be confirmed or outruled by doing concentration response studies in normal and diseased tissues in the presence of a constant concentration of diphenhydramine.

Histamine may be released from gallbladder tissue as part of the inflammatory reaction in cholecystitis and may be involved in the control of normal gallbladder motility. The results of this study suggest that the involvement of histamine in the control of gallbladder motility may depend upon the severity of cholecystitis.

Histamine H_1 and H_2 receptors have been described in guinea pig and baboon gallbladders (185,126). The importance of histamine H_2 receptors in the control of gastric acid secretion is well known, but there have been few investigations of the effects of histamine receptor stimulation on human gastrointestinal motility. Relaxation of the human lower oesophageal sphincter following H_2 receptor stimulation has been described (186). In this study the predominant effects of histamine on human gallbladder was an H_1 receptor-mediated contractile effect. A very small H_2 receptor-mediated component was observed in less than half (10 out of 23) of the gallbladders studied. This is in marked contrast to results using guinea pig gallbladder where a major H_2 receptor mediated component was demonstrated (124,185). The potent H_2 receptor antagonist, cimetidine, did not alter the sensitivity or the maximal contractile

responses of human gallbladder strips to histamine. Also, histamine H_2 receptor-mediated relaxation of CCK-OP-induced tone (measured in the presence of diphenhydramine), can be easily demonstrated in guinea pig gallbladder strips (185). In human gallbladder strips, however, under similar conditions, no H_2 receptor-mediated component was observed. Further evidence for a lack of functional histamine H_2 receptors in human gallbladder comes from the results with selective histamine H_2 receptor agonists. Impromidine, which possesses less than 0.001% of the agonist activity of histamine at H_1 receptors and up to 50 times the activity of histamine at H_2 receptors (187), failed to elicit any responses in human gallbladder. Another so-called H_2 selective agonist 4-methylhistamine, also failed to relax human gallbladder strips in normal Krebs, but in the presence of diphenhydramine small relaxations were seen. It was approximately 100-fold weaker than histamine in producing H_1 mediated contractile responses.

Thus, while there is a significant population of H_2 receptors in the guinea pig gallbladder, there is but a small population demonstrable in the human gallbladder, where they are probably physiologically unimportant.

HORMONAL REGULATION

Cholecystokinin

Findings in this study confirm that the human gall-

contracts in a concentration-related fashion to cholecystokinin. With increasing CCK-OP concentration the onset of action was quicker but surprisingly the time required to develop maximal tension increased taking up to nine minutes to reach maximum with a CCK-OP concentration of 1 μM. The prolonged relaxation time, 45 minutes for maximal contraction and lack of effect at washing are previously unreported in vitro. These findings are in keeping with in vivo results where the gallbladder continues to empty for up to 30 minutes after CCK administration (188).

As in other studies (70,81) the cholecystokinetic effect was unaltered by the antimuscarinic agent atropine. Histamine H_1 and H_2 receptor antagonists did not alter the sensitivity or contractile response to CCK-OP; this is in contrast to the findings in the guinea pig (124) where histamine H_2 receptor blockade augmented the CCK contractile effect. As only a very small histamine H_2 receptor population was demonstrated in the gallbladder in this study it is not unexpected that H_2 receptor antagonism did not effect the CCK-OP response.

The antagonistic effect of dbGMP in the CCK-OP contraction (85) was confirmed. The nature of this inhibition is still unclear as indeed is the mechanism of action of cholecystokinin itself. Increased levels of cyclic GMP have been noted in association with CCK activity (82) but while some authors believe this to be unimportant (83)

Longinov (84) stated that cyclic GMP is presquisite for the CCK contractile effect. That CCK causes an elevation in cyclic GMP and its contractile effect is inhibited by dbGMP would seem to suggest that cyclic GMP plays some regulatory role in the initiation or maintenance of the CCK contractile effect. In analysing the inhibiting effect of the dbGMP most authors (87) believe it acts as a competitive inhibitor, while Miller et al (86) believe it acts by interacting with the cholecystokinin molecule.

It seems reasonable to postulate that cyclic GMP is a mediator in the CCK effect and that this is the agent, because of its chemical similarity, which is antagonised by dbGMP.

Gallbladder Sensitivity and Contractility to CCK-OP

Results of this study failed to show any difference in sensitivity to cholecystokinin between normal gallbladders and those with acalculous or calculous cholecystitis. This is in marked contrast to results in in-vivo studies which suggest altered gallbladder sensitivity in both calculous (165,168) and acalculous (170,171) cholecystitis.

In these in-vivo studies gallbladder sensitivity to cholecystokinin is assessed by the change in gallbladder volume in response to cholecystokinin which is infused or endogenously released in response to a meal. Three methods are used to measure gallbladder volume:-

1. It is calculated on films from a standard oral cholecystogram.

2. It is calculated from gallbladder dimensions as measured by ultrasonography.
3. Percentage emptying is measured by the decrease in radioactivity over the gallbladder area during a technetium scan.

These methods may accurately assess the changes in gallbladder volume which may, or may not, reflect gallbladder sensitivity to cholecystokinin. Changes in volume are not due to gallbladder response to cholecystokinin alone. Gallbladder emptying is also affected by the resistance to flow through the cystic duct, the common bile duct pressure, and sphincter of Oddi activity. Increased cystic duct resistance has been reported in association with gallstones and is present even in the presence of lithogenic bile (17): this would inhibit gallbladder emptying and could be interpreted as a decrease in gallbladder sensitivity to cholecystokinin. The cystic duct does contract in response to cholecystokinin but is less sensitive than the gallbladder in normal and diseased states (180) and it is not known if, under physiological circumstances, it does contract. Exogenously administered CCK may give unphysiological levels of CCK causing cystic duct contraction, thereby increasing resistance to outflow and inhibiting gallbladder emptying. This too, would be interpreted as a decreasing gallbladder sensitivity to cholecystokinin.

In this study there was a significant difference in the contractility in the disease groups. The acalculous (cholesterolosis) and chronic mild cholecystitis groups had contractile responses greater than normal. However, due to the small number of normal and acalculous specimens this difference was not significant. This increased contractility probably reflects the muscle hypertrophy noted in these groups and may be responsible for the increased "sensitivity" to cholecystokinin reported in-vivo studies. It may also represent the "hypertrophic" cholecystosis (acalculous cholecystitis) group reported by Goldstein et al which (145) had greater than 80% emptying of gallbladder in response to cholecystokinin.

Thompson et al (167) described a group of 'non-contractors'- a group of patients whose gallbladders did not respond to cholecystokinin; these are possibly represented in this study by the chronic advanced cholecystitis group which contracted very poorly, the responses were significantly less than in normal, chronic mild, and cholesterolosis groups. Unfortunately in Thompson et al's study the histological state of the gallbladder was not assessed and related to gallbladder contractility.

The relatively poor contractility of the chronic advanced cholecystitis group is surprising as the degree of muscle hypertrophy was similar to that in the chronic mild cholecystitis group. It may be due to the fibrosis in the muscle: as there was no change in the sensitivity, the

difference in contractility does not represent any change in cholecystokinin affinity for the receptors. The finding of poor contractility in the chronic advanced and moreso in the acute cholecystitis groups differs from that of Goldberg et al (177) who found that the chronically and acutely inflammed canine gallbladder have the same contractility as normal. However, in this study the acute inflammation in the gallbladder was superimposed on severe chronic cholecystitis with marked muscle fibrosis and destruction, whereas the cholecystitis in Goldberg et al's study was chemically induced acute cholecystitis. Therefore, the results are not really comparable.

In the ground squirrel fed a lithogenic diet Fridhandler et al. (164) reported no change in sensitivity but decreased contractility to cholecystokinin. However, the muscle thickness was not measured, gallstones did not form and the lithogenic index did not reach I.

In summary there is no change in sensitivity to cholecystokinin in calculous or acalculous gallbladder disease and the findings in this study are not in disagreement with in-vivo studies as, strictly speaking, those studies measured gallbladder contractility rather than sensitivity. The changes in contractility noted in this study help explain some of the findings in-vivo studies.

Gastrin, Pentagastrin and Caerulein

The cholecystokinetic effect of gastrin, pentagastrin

caerulein were confirmed in this study. As each of these peptides and cholecystokinin has an identical carboxyl terminal pentapeptide-amide which contains the cholecystokinetically active part of the molecule, it is not surprising that the characteristics of the contractile effects of these agents are similar. That the effects of caerulein, gastrin and pentagastrin are antagonised by dbGMP which is a specific cholecystokinin antagonist, confirms that they act at the same receptor site as cholecystokinin.

Gastrin and pentagastrin were equipotent and efficent on a molar basis and were approximately 100 times less potent than cholecystokinin octapeptide, which is 10 and 180 times more than previously reported for gastrin (189) and pentagastrin (80) respectively. It is not known if the sulphated gastrin II is a more potent cholecystokinetic agent than gastrin I (89,90). Sulphation of the tyrosyl residue is known to be essential to the cholecystokinetic activity of cholecystokinin (70) and caerulein (96). However, as the sulphation of cholecystokinin and caerulein is at position 7 and sulphation of gastrin II is at position 6, it may or may not affect the gastrin II cholecystokinetic potency.

It is clear that gastrin has a cholecystokinetic effect which may be physiological, particularly if gastrin II is more potent than gastrin I.

Caerulein has been reported to be a more potent

cholecystokinetic agent than cholecystokinin (80) but in this study was found to be 25 times less potent than cholecystokinin-octapeptide on a molar basis. As a maximal contractile effect was not achieved, it is not possible to comment on its efficacy.

Vasoactive Intestinal Polypeptide (VIP)

Although VIP has been shown to affect motility in a variety of gastrointestinal tissues (190,191) this study failed to demonstrate any VIP-mediated effect on human gallbladder contractility *in vitro*. The lack of effect of VIP on human gallbladder contractility is surprising as it is believed to be the cause of dilation of the gallbladder (106) which occurs in association with W.D.H.A. syndrome (107). It was decided to test the efficacy of the VIP preparation in a test system previously shown to respond to VIP; namely guinea pig gallbladder. VIP from three sources was studied; one 'natural' (porcine) and two 'synthetic' and each preparation produced similar biphasic effects on guinea pig gallbladder strips. At low concentrations, a concentration-dependent contractile effect was seen which was maximal at 100 pM. At higher concentrations (100 nM to 1 μM) concentration-dependent relaxations were seen. All previous reports on guinea pig gallbladder muscle show VIP to be a potent relaxant of both resting, and CCK-induced tone (102,103). However, in other gastrointestinal tissues, e.g. ileum (192,193) and

duodenum (192) VIP causes contractile responses. In one of these studies (193), the contractile effect was partially antagonised by atropine. In this study, the contractile responses were unaffected by atropine, diphenhydramine or indomethacin, suggesting that the VIP contractile effect is not mediated via muscarine or histamine receptors or via stimulation of prostaglandin biosynthesis.

Natural VIP may contain a CCK-like contaminant (194). However,the contractile responses in this study were caused by both 'synthetic' and 'natural' VIP. Also, should CCK be present in the VIP preparations, contractile responses in human gallbladder strips should have been observed. It is unlikely, therefore, that the contractile responses were due to a CCK-like contaminant.

VIP and the GI hormone, secretin, are structurally similar (195). Secretin has been reported to cause gallbladder smooth muscle contraction (98) and to augment CCK-OP induced contractions (100). VIP may cause gallbladder contractions by a similar mechanism. In a previous report (103), VIP, at a concentration of 10 ng/ml (approximately 3 pM), abolished all spontaneous activity. In this study spontaneous activity was only temporarily affected during the acute rise or fall phase in tension caused by low and high concentrations respectively. VIP concentration-related reductions of tone have been previously reported; 1-200 ng/ml (300 pM - 60 nM approximately) caused 5 - 61% reductions in resting tone. Maximum contractile responses occured with

100 pM VIP in the present study and relaxant responses were observed only at much higher concentrations.

The most pronounced action of VIP is the stimulation of water and electrolyte secretion by small and large intestine (101, 196) and it also has been shown to reverse the direction of gallbladder water and electrolyte transport (197). As the gallbladder exhibits the property of receptive relaxation it is reasonable to postulate that the gallbladder dilation noted in patients with W.D.H.A. is a result of the secretory effect of VIP rather than an effect due to a direct VIP-induced smooth muscle relaxation.

VIP has been proposed as a possible mediator in non-adrenergic-noncholinergic relaxation (62) and as a possible regulator of receptive relaxation in the human gallbladder. Findings in this study suggest nonadrenergic-noncholinergic inhibition does not occur in the human gallbladder and furthermore VIP does not have a relaxant effect. The primary function of the VIP in human gallbladder nerve endings (105) may be the regulation of gallbladder secretion.

Substance P

This is the first report of the in-vitro effects of substance P on gallbladder motility where it caused concentration-related contractile responses. Of the agents studied substance P had the slowest onset of action, taking more than one minute to cause contractile responses.

Unlike the intestine, where the contractile effect of substance P is partly antagonised by atropine, the effect in the gallbladder was unaffected by atropine, histamine receptor antagonists, indomethacin or the selective

cholecystokinin antagonist dbGMP. This suggests that substance P acts on specific receptors:- Lee (198) has reported multiple substance P receptor types. It is reasonable to postulate a role for substance P in the regulation of biliary motility as recently Cai and Gabella (23) have reported substance P in nerve endings in the wall of the guinea pig gallbladder. In this study, however the maximal substance P-produced contractile responses were less than 20% of those produced by cholecystokinin-octapeptide which suggests that it is not an important regulator of gallbladder contraction.

Like vasoactive intestinal polypeptide substance P has been identified in the gallbladder wall nerve endings but its role if any in the regulation of gallbladder motility remains speculative.

Secretin and other Hormones

A number of hormones have been reported to influence gallbladder motility, but in this study were found to have no effect on resting or cholecystokinin-induced tension in gallbladder strips.

Early reports of secretin-induced contractile responses in vitro (91) are believed to have been caused by cholecystokinin contamination of the secretin preparation: Indeed in the early experiments in this study the secretin preparation used caused contractile responses which were found to be due to cholecystokinin contamination. Some in-vivo studies have shown an increase in gallbladder

pressure (98) and augmentation of cholecystokinin-induced pressure (100) while others have reported a relaxant effect on gallbladder pressure (92). It is possible that the in-vivo findings were due to indirect effects as is suggested by the lack of effect of secretin in this study.

The lack of effect of glucagon on gallbladder strips in this study is in agreement with reports from previous in-vitro studies in human (91) and guinea pig gallbladder. This suggests that the reports of gallbladder relaxation in-vivo (109) with pharmacological doses, was an indirect effect.

In this study motilin had no effect on gallbladder strips which confirms reports from previous studies (117). Adrian (116) in in-vivo studies reported that motilin was almost equipotent with cholecystokinin in causing gallbladder contraction. Itoh et al (36) have shown that in the fasting state, the gallbladder contracts in synchrony with the migrating myoelectric complex which has been shown to be, at least partly, regulated by motilin (115). This suggests that the gallbladder response to motilin is secondary to the migrating myoelectric complex and is indirect.

Motilin may regulate gallbladder motility in the interdigestive period by an indirect effect.

This in the first in-vitro study of the effect of bombesin on the human gallbladder and it shows that it has

no direct effect on gallbladder motility. This is in keeping with the in-vivo studies (122) which suggested that its effects on the biliary system were secondary to the release of cholecystokinin by infused bombesin.

In-vivo studies have shown somatostatin to inhibit the gallbladder's contractile response to vagal stimulation (118) but in this study somatostatin did not inhibit resting or cholecystokinin-induced tension. This suggests that the inhibitory effect may be indirect.

There have been no previous reports of the effects of neurotensin or vasopresin on gallbladder motility and in this study these agents were found not to affect gallbladder strips.

Recently Cox et al (199) reported that, in fasting human serum, the principal cholecystokinetic effect is due, not to cholecystokinin, but, to small peptides that act on receptors other than cholecystokinin receptors. As the gastrins probably act on the cholecystokinin receptors, only one such peptide was identified in the present study, i.e. substance P. In view of the multiple substance P receptor subtypes (198) the present findings are consistent with those of Cox et al (199).

CONCLUSIONS

Acalculous cholecystitis and cholesterol gallstone disease are probably closely related diseases with a multifactorial etiology, cystic duct resistance to bile flow is probably a common etiological factor.

It has been suggested that gallbladder sensitivity to cholecystokinin is altered in calculous and acalculous cholecystitis but this is found not to be so in this study. The increased contractility reported in in-vivo studies in patients with gallstone disease is probably secondary to the muscle hypertrophy.

Vasoactive intestinal polypeptide is not as potent a gallbladder relaxant as was formerly believed. Apart from cholecystokinin and gastrin substance P is the only GI hormone which has a direct effect on gallbladder motility where it causes a concentration-related contractile response. As substance P has been reported in nerve-endings in the gallbladder this may represent a function for this hormone in gallbladder motility.

Gallbladder sensitivity to histamine is altered in cholecystitis, increasing with increasing degrees of inflammation in the gallbladder.

Adrenergic nerve stimulation causes relaxation of gallbladder which is mediated by β_2-adrenergic receptors. The human gallbladder contains only a very small population of α-adrenergic receptors which mediate contraction.

In this study non-adrenergic, non-cholinergic inhibition was not demonstrated in human gallbladder.

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ABSTRACTS:

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2. Feeley, T.M., Clanachan, A.S. Histamine Receptors in The Human Gallbladder and Cystic Duct. Br. J. Clin. Pharmac. 1983, in Press.

PUBLICATIONS:

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7. Feeley, T.M., O'Higgins, N. 1983. An Atypical Case of Abdominal Actinomycosis. Irish. Med. J. 76:387.

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Submitted

10. Feeley, T.M., Rosenberg, I.L. Malignant change in a fistula in Ano. (J.Roy.Coll.Surg.Edin.).

11. Lennon, F., Feeley, T.M., Clanachan, A.S., Scott, G.W. The Effects of Histamine Receptor Stimulation in the Human Gallbladder. Gastroenterology.

PRESENTATIONS TO LEARNED SOCIETIES:

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4. Clanachan, A.S., Feeley, T.M. September 1983. Histamine receptors in the human gallbladder and cystic duct. Annual meeting of British Society of Pharmacology (Clinical Section) Galway, Ireland.